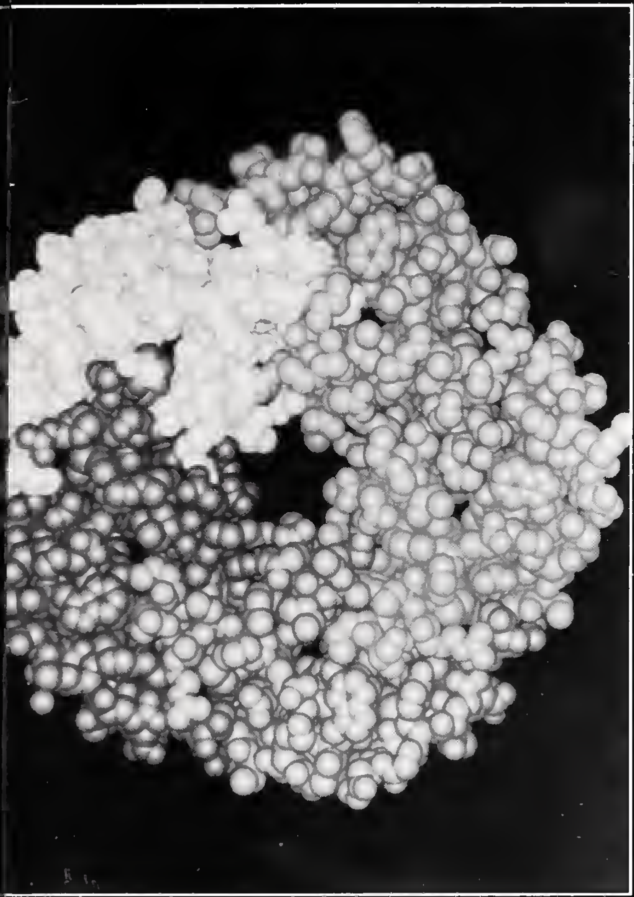
Program Highlights 1991

National
Center for
Research
Resources,
NIH



Annual
Report

U.S. DEPARTMENT
OF HEALTH AND
HUMAN SERVICES
Public Health Service
National Institutes
of Health

National Center for Research Resources

The National Center for Research Resources (NCRR) supports NIH intramural and extramural biomedical research that includes clinical research studies, development and studies of animal and other models of human diseases or disorders, basic biomedical research, and instrumentation research. NCRR also supports the operation of instrumentation and computer facilities as well as research centers in academic institutions with large minority enrollments. In addition, NCRR provides the NIH intramural scientific community with library services and medical arts and photography services. The activities and services of the NCRR—and publications that describe them—are listed on this page. For more detailed information about particular NCRR programs or the resources they support, contact the program directors at the numbers listed on this page.

NCRR Extramural Research Resources comprise seven programs:

- **Biological Models and Materials Research Program.** Telephone: (301) 402-0630;
- **Biomedical Research Support Program.** Telephone: (301) 496-6743;
- **Biomedical Research Technology Program.** Telephone: (301) 496-5411;
- **Comparative Medicine Program.** Telephone: (301) 496-5175;
- **General Clinical Research Centers Program.** Telephone: (301) 496-6595;
- **Research Centers in Minority Institutions Program.** Telephone: (301) 496-6341;
- **Research Facilities Improvement Program.** Telephone: (301) 496-5411.

NCRR Intramural Research Resources comprise two programs and two branches:

- **Biomedical Engineering and Instrumentation Program.** Telephone: (301) 496-4741;
- **Library Branch.** Telephone: (301) 496-2447;
- **Medical Arts and Photography Branch.** Telephone: (301) 496-2868;
- **Veterinary Resources Program.** Telephone: (301) 496-2527.

NCRR Publications:

- The *Research Resources Reporter* is a monthly newsletter that describes NCRR-supported biomedical findings and research in progress at universities, medical centers, and other research institutions across the United States. The *Reporter* also describes research services available to biomedical investigators and publishes a question-and-answer column relating to research resources.
- The NCRR *Program Highlights* is an annual report of research accomplishments and significant services of each NCRR program and branch during the fiscal year. Detailed budget information about NCRR is also provided.
- The NCRR *General Clinical Research Centers Directory* contains information about General Clinical Research Centers, their staffs, resources, and major areas of investigation. Investigators who receive their primary research funding from the National Institutes of Health or the Alcohol, Drug Abuse and Mental Health Administration are eligible to perform clinical research studies at one of the more than 70 centers.
- The NCRR *Biomedical Research Technology Resources Directory* lists resource centers supported by the Biomedical Research Technology Program, their staffs, resources, and major areas of investigation. The facilities are dedicated to applying the latest advances in the physical sciences, mathematics, computer science, and engineering to biomedical research. The facilities and their services are available to qualified investigators.
- The NCRR *Resources for Comparative Biomedical Research Directory* contains information about specialized animal research and supply facilities, their staffs, types of resources, and major areas of research. The resources range from breeding facilities for lower animal forms to nonhuman primate research centers. These facilities and their services are available to qualified investigators.
- The NCRR *Biological Models and Materials Research Resources Directory* lists centers that study nonmammalian models for biomedical research—cells, lower organisms, computer simulations, and mathematical models—and resource centers that provide critical biomaterials. Nonmammalian organisms, viruses, bacteria, fungi, cell lines, human tissue, DNA probes, and chromosome libraries are available. The directory also lists the staffs, types of research, and major areas of investigation at each center. These facilities and services are available to qualified investigators.

All NCRR directories are updated and reissued approximately every 15 months.

Single copies of NCRR publications may be obtained free of charge from the Research Resources Information Center, 1601 Research Boulevard, Rockville, MD 20850. Telephone: (301) 251-4970.

Queries about the NCRR may be directed to the Office of Science and Health Reports, National Center for Research Resources, 5333 Westbard Avenue, Westwood Building, Room 10A15, Bethesda, MD 20892.
Telephone: (301) 496-5545.

National
Center for
Research
Resources

Program Highlights 1991

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Annual
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U.S. DEPARTMENT
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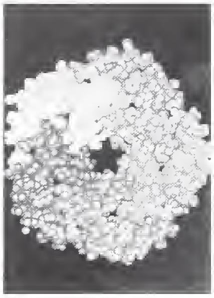
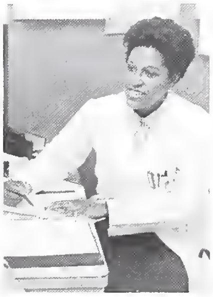


The three-dimensional structure of the cholera toxin has been determined by scientists at the NCRR-supported CCD Area Detector Research Resource in Argonne, Illinois. Detailed knowledge of the toxin's shape may aid in the development of improved vaccines or drug therapies.

Edwin Westbrook, Argonne National Laboratory

Dr. Michele Sartori, clinical instructor of medicine at Baylor College of Medicine in Houston, Texas, is investigating the use of laser-induced fluorescence in diagnosing the extent and type of atherosclerosis, with the aid of the NCRR-funded computerized database management and analysis system (CDMAS).

Dr. M. Sartori



Dr. Joyce Harp, assistant professor of medicine at Emory University School of Medicine in Atlanta, Georgia, was one of three researchers to receive the first Minority Clinical Associate Physician (MCAP) awards presented by the General Clinical Research Centers Program. Dr. Harp is studying the relationships between hypertension and obesity.

Eddie Jackson, Emory University

Rhesus macaques are among the many species of nonhuman primates that are reared and studied at the seven Regional Primate Research Centers supported by the National Center for Research Resources.

Vince Warren, Oregon Regional Primate Research Center

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A Brief Preface to the 1991 Annual Report of the National Center for Research Resources

The National Center for Research Resources (NCRR) of the National Institutes of Health (NIH) supports biomedical research nationwide. The Center administers and manages seven extramural programs, two intramural programs, and two intramural branches.

The Extramural Research Resources programs support the development of facilities and resources that enhance biomedical research and provide research training on an institutional, regional, or national basis. These programs include the General Clinical Research Centers Program, Biomedical Research Technology Program, Comparative Medicine Program, Biomedical Research Support Program, Biological Models and Materials Research Program, Research Centers in Minority Institutions Program, and Research Facilities Improvement Program.

Appropriations for the extramural programs administered by NCRR in fiscal year 1991 amounted to $335.8 million, from which the Center made awards totaling $322.4 million. The remainder of the funds provided the technical and personnel support needed to administer the programs.

The Intramural Research Resources programs and branches offer essential services and resources to the NIH intramural research community. These programs and branches comprise the Biomedical Engineering and Instrumentation Program, Library Branch, Medical Arts and Photography Branch, and the Veterinary Resources Program.

The intramural programs and branches are supported by the NIH Management Fund (a small percentage of the funds appropriated for the NIH intramural research programs) and by the NIH Service and Supply Fund (a fee-for-service mechanism). In fiscal year 1991 the NCRR Management Fund totaled $21.3 million, and the Service and Supply Fund income was $34.0 million.

A more detailed breakdown of NCRR funding for 1991 appears in the tables preceding both the extramural and the intramural sections of this annual report.

The following pages of *Program Highlights 1991* describe the mission, scope of support, and significant accomplishments of each program or branch during the year.

Robert A. Whitney, Jr., D.V.M.
Director, National Center for Research Resources
National Institutes of Health

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Overview of Extramural Research Resources Activities

National Center for Research Resources Overview of Extramural Research Resources FY 1991 Activities

Program	Funding	Major Resources Supported
General Clinical Research Centers Program	$ 118,446,000	• 74 General Clinical Research Centers • 45 Clinical Associate Physicians • 3 Minority Clinical Associate Physicians • 1 small business innovative research grant • Computerized Database Management and Analysis Systems (CDMAS) • cofunded MBRS grant to Meharry Medical College GCRC • cofunded Science Education Partnership Award • cofunded contract to study career paths for clinical researchers
Biomedical Research Technology Program	$ 40,880,000	• 58 Biomedical Research Technology Resource Centers • 6 research project grants • 5 resource-related project grants • 22 small business innovative research grants • 11 small grants • PROPHET computer network
Comparative Medicine Program	$ 68,482,000	• 7 Regional Primate Research Centers • 34 Laboratory Animal Research Resource Centers • 18 research project grants • 9 resource-related research project grants • 46 animal facility improvement awards • 11 research career awards in laboratory animal sciences • 17 postdoctoral research training programs • 3 small business innovative research grants • Chimpanzee Breeding and Research Program • Specific-Pathogen-Free Rhesus Monkey Breeding and Research Program
Biomedical Research Support Program	$ 64,458,000	• Biomedical Research Support Grants awarded to 628 institutions • 139 Shared Instrumentation Grants • 391 Minority High School Student Research Apprentice Program awards to support 2,970 students and 468 teachers • 12 Science Education Partnership Awards
Biological Models and Materials Research Program	$ 8,058,000	• 4 Biological Models Research Centers • 20 research project grants • 2 resource-related research projects • 5 resource facilities
Research Centers in Minority Institutions Program	$ 22,090,000	• 17 RCMI awards
Total Extramural	$ 322,414,000*	

*In addition, $2,748,000 of reimbursable authority from other Federal agencies was obligated by NCRR for FY 1991.

General Clinical Research Centers Program

Program Description

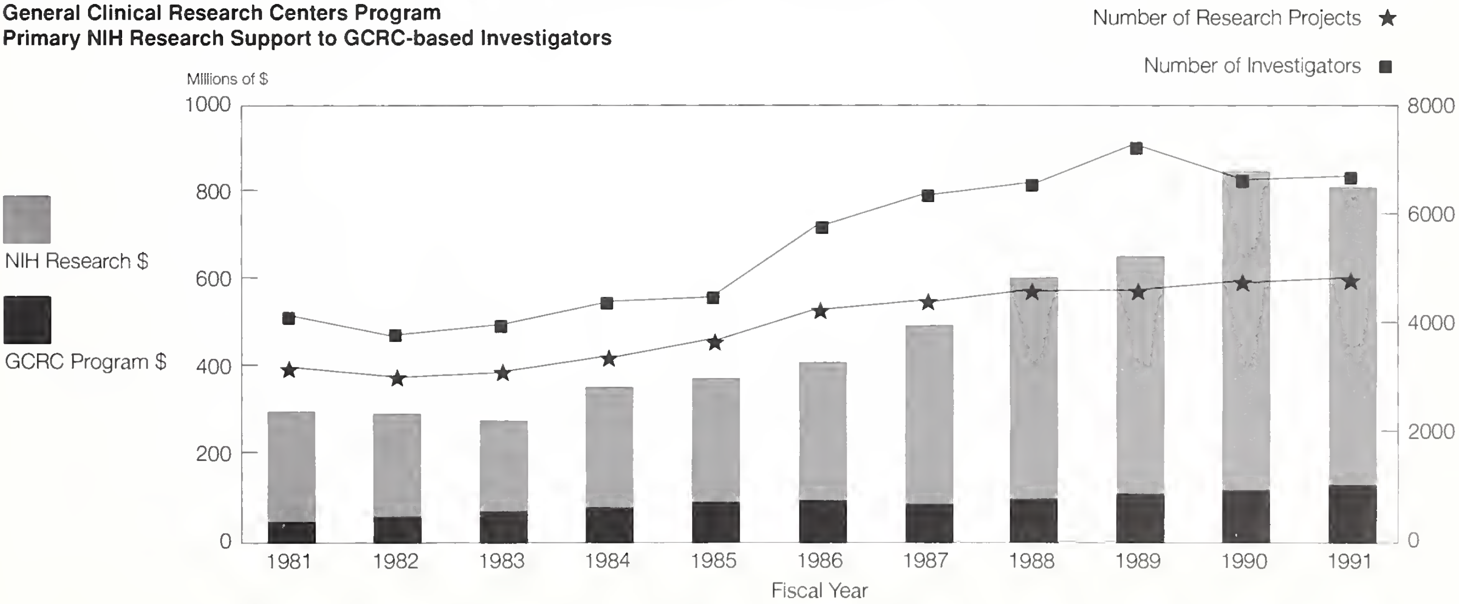
The General Clinical Research Centers (GCRC) Program of the National Center for Research Resources significantly expands opportunities for biomedical achievements by clinical investigators who receive their primary research funding from other components of the National Institutes of Health (NIH) and from the Alcohol, Drug Abuse, and Mental Health Administration (ADAMHA). In fiscal year 1991 GCRC Program funding supported the network of GCRC's and other related activities with $118,446,000.

Investigators who use the resources across the 30-state network of GCRC's continue to be at the forefront of biomedical research, evidenced by the level of primary research support they receive from other NIH programs. The 74 GCRC-funded centers in the program's national network extend the range and intensity of research possible by complementing the funding provided by the NIH and ADAMHA to grantees who study cancer, heart diseases, diabetes, aging, alcoholism, drug addiction, arthritis, and infectious diseases; develop gene therapies; and investigate scores of other health problems affecting children and adults at GCRC sites. This year 6,477 investigators carried out 4,758 projects. Research investigations mirror the research missions of the other institutes and centers of the NIH and ADAMHA. The centers offer a research infrastructure, often otherwise unavailable, that includes specialized research nurses, research dieticians, biostatisticians, computer hardware and software systems for data management and analyses, sophisticated laboratories, and customized facilities and professional staffing for inpatient and outpatient research.

Conceptually the GCRC Program is analogous to a 600-bed research-intensive hospital distributed geographically to reflect the population density of the continental United States. It constitutes the extramural NIH counterpart of the Warren Grant Magnuson Clinical Center on the NIH campus in Bethesda, Maryland. In FY 1991 the program accommodated approximately 100,000 research patient days, 31,000 admissions, and 200,000 outpatient research visits that ranged in length from a half-hour to 14 hours.

To ensure that the nation has a continuing supply of physicians and dentists trained to be independent clinical investigators, since 1974 the GCRC Program has sponsored the Clinical Associate Program (CAP) as a competitive supplement to GCRC grants. The CAP provides up to 3 years of support to a funded physician or dentist at a salary commensurate with those at the GCRC grantee institution. To date, 216 have graduated from CAP. In 1991 the GCRC initiated a Minority Clinical Associate Program (MCAP) to attract

General Clinical Research Centers Program
Primary NIH Research Support to GCRC-based Investigators



underrepresented minority physicians and dentists to clinical research at GCRC sites. Minority candidates may apply for either the CAP or MCAP awards. Several MCAP awards were made in FY 1991 for this new program.

To honor GCRC principal investigators whose potential in clinical research has already been proved, the GCRC Program annually presents an Award for Excellence in Clinical Research. In 1991 Dr. John D. Brunzell, professor of medicine at the University of Washington, Seattle, received the third of these annual prizes. An authority on the process by which fats, cholesterol, and triglycerides are transported and used by the body, Dr. Brunzell has greatly contributed to scientific knowledge about familial combined hyperlipidemia, a genetic disorder that is one of the two most common precursors of heart attack in middle-aged men.

During 1991 the U.S. Department of Health and Human Services issued an important new statement of objectives, *Healthy People 2000*. The document stressed personal responsibility as a key to good health—with each individual practicing enlightened behaviors that maintain fitness and avoiding actions or habits that lead to chronic and acute illness. A correlated responsibility emphasized in the document is society's need to extend the benefits of good health to its most vulnerable members.

The NIH is directing significant resources toward health promotion and disease prevention strategies, tackling a full range of preventable health problems, and attempting to develop health research strategies that will benefit the vulnerable populations identified in *Healthy People 2000*. The remainder of this GCRC report will highlight research progress made during 1991 that may help to advance national health promotion and disease prevention objectives.

Research Highlights

AIDS

Immunoglobulin Reduces Bacterial Infections in Pediatric AIDS. In a multicenter, double-blind, controlled study that included investigators at the Baylor College of Medicine GCRC in Houston, Texas, children treated with intravenous immunoglobulin (IVIG) had significantly fewer serious bacterial infections than did pediatric AIDS patients treated with a placebo.

The study, which started in March 1988, enrolled 372 symptomatic HIV-infected children at 28 centers; because of benefits seen in the IVIG-treated children, the study was terminated in January 1991.

Of the 187 children treated with placebo, 30 had developed one or more serious bacterial infections; only 18 of the 185 IVIG-treated children did so. The most prevalent bacterial isolate was *Streptococcus pneumoniae*.

Drug Treatment of AIDS and Associated Conditions. Under the auspices of the NIH-funded AIDS Clinical Trials Group, GCRC's are devoting significant efforts and resources to research on treating AIDS and associated opportunistic infections.

Drugs currently under study include zidovudine (azidothymidine or AZT); interferon; interleukin-2; granulocyte-macrophage colony-stimulating factor; recombinant soluble CD4 (receptin); 2',3'-dideoxyinosine (ddI), dideoxycytidine (ddC), 3'-azido-2',3'-dideoxyuridine (AzdU); 3'-deoxy-3'-fluorothymidine; acyclovir; pentamidine; spiramycin; ganciclovir; gamma globulin; trimethoprim; sulfamethoxazol; trimetrexate; foscarnet; clindamycin; primaquine; and itraconazole.

Many of the studies have progressed from phase 1 clinical evaluations to phases 2 and 3; some have moved from the carefully controlled conditions of the GCRC's to wider clinical testing at many medical centers throughout the United States.

Newer Agents Tested Against Most Common Fatal Infection in AIDS. Investigators at the Indiana University GCRC in Indianapolis participated in a multicenter nonrandom pilot study of a combination regimen of clindamycin and primaquine, two relatively new drugs, against *Pneumocystis* infection in AIDS patients. The pilot study, which was sponsored by the NIH AIDS Clinical Trials Group, was designed to assess the safety and efficacy of clindamycin and primaquine in treating mild or moderately severe infections.

Of the 36 patients treated, 33 had a favorable response. Even though most of the patients developed a rash, 28 were able to complete the course of therapy. Since *Pneumocystis* pneumonia is often fatal, the identification of effective treatment is encouraging.

***Study of Lung Macrophage Receptors for* Pneumocystis carinii.** Previous research indicated that naturally defensive cells called macrophages could destroy the microorganism *P. carinii* inside lung tissue. More recently GCRC investigators at the Children's Hospital of Boston found that the macrophages active against *P. carinii* had a glycoprotein receptor for the microorganism that contained a high concentration of the carbohydrate mannose.

Dr. Timothy Cooley (at right), of the GCRC at Boston University School of Medicine in Massachusetts, examines a patient participating in a multicenter clinical evaluation of the anti-AIDS drug 2',3'-dideoxyinosine, or ddI. The drug, which mimics a natural building block of DNA, interferes with the reproductive cycle of the AIDS virus.

David Keough, Boston University School of Medicine

The scientists found that human and rat macrophages bound 90 percent fewer cultured *Pneumocystis* organisms in the presence than in the absence of competitive inhibitors of mannose receptor activity. The studies have established that the macrophage receptor is necessary for *P. carinii* attachment to these cells; they emphasize the role of the alveolar macrophage in first-line defenses against *Pneumocystis* infections.

Cancer

Breast Cancers Imaged by Positron Emission Tomography. During 1991 investigators at the University of Michigan GCRC in Ann Arbor reported on the feasibility of positron emission tomography (PET) scans to identify both primary and metastatic cancers of the breast. In a study of 12 patients, all 10 primary breast cancers were imaged by PET after intravenous administration of a radioactively labeled glucose analogue 2-[fluorine-18]-fluoro-2-deoxy-D-glucose (FDG).

All 10 bone metastases and all 5 known soft tissue metastases were visualized, and 4 previously unsuspected lymph node lesions were discovered. In two women with negative mammograms due to dense breast tissue, FDG PET scans undertaken in this preliminary study defined primary cancers of the breast.

The investigators also explored the feasibility of using the FDG PET scan method to monitor the responses of six breast cancer patients to chemotherapy and hormonal therapy. A rapid decline in uptake of the FDG marker was observed in the patients' cancers following 21 days of treatment with a combination of cytoxan, adriamycin, estrogen, methotrexate, and 5-fluorouracil. In several instances declines in FDG uptake preceded reduction in the size of the tumor.

These preliminary data indicate that FDG PET scanning can image primary and metastatic breast cancers and can monitor their response to therapy, often detecting metabolic change before anatomic changes occur. The GCRC investigators indicate, however, that long-term followup of these patients is essential to determine the significance of the early metabolic changes they detected.

Evidence of a Gene for Proliferative Breast Disease. GCRC-supported scientists at the University of Utah in Salt Lake City reported that proliferative breast disease (PBD), a significant risk factor for the development of breast cancer, is an inherited trait in families that have a high prevalence of either premenopausal or postmenopausal breast cancers.

The investigators studied 20 kindreds, or extended families, in which at least two first-degree relatives (such as mother, sister, or daughter) had breast cancer. Women who were not genetically related but were spouses of male members of the families participated as control subjects. Cytogenetic analysis of tissue aspirated from the breasts of all women in the study revealed proliferative breast disease in 35 percent of the clinically normal first-degree relatives of the women who had breast cancer but in only 13 percent of the control women.

Further genetic analysis of the aspirated breast tissue will be performed on cultured cells in the university's GCRC core laboratory.

Targeting of Neuroendocrine Cancers of the Stomach and Pancreas. Investigators at Ohio State University GCRC in Columbus and their colleagues have found that octreotide acetate, an analogue of somatostatin—which inhibits release of growth hormone from the pituitary—may be used to specifically target tumors during surgery. After predicting that tumors of the stomach and pancreas would have receptors for somatostatin and therefore would bind octreotide acetate, the investigators were successful in developing a radioiodine-labeled octreotide marker to define neuroendocrine tumors before and during surgery. In this way they have been able to localize noninvasively a gastrinoma metastatic to the stomach, a vasoactive intestinal polypeptide-secreting tumor that metastasized to the liver, and lymph node metastases in a patient with carcinoid syndrome.

This novel diagnostic approach is proving useful in patients whose tumors have receptors for somatostatin. It is also the first time that radiolabeled receptors have been used to guide surgical procedures for cancer, the investigators note.

Chronic Disabling Conditions

Osteoporosis Linked to Thyroxine Treatment in Menopausal Women. A study of bone mineral density in 19 menopausal women at the Temple University GCRC in Philadelphia, Pennsylvania, showed that long-term treatment with L-thyroxine is associated with decreased density of the spine and hip.

The Philadelphia scientists used a technique called dual photon absorptiometry to measure bone mineral density in the 19 thyroxine-treated women and in control women who had not received thyroxine. They found that the treated group had bone densities in the lumbar spine, the neck of the femur, and a hip area known as Ward's triangle that were 11, 9, and 17 percent lower, respectively, than the bone densities of untreated control women. Seven of the 19 women had been hyperthyroid as young adults; these 7 had significantly reduced bone density in the hip.

It had previously been thought that thyroxine doses slightly higher than normal physiologic levels were not harmful, and that a slight suppression of thyroid-stimulating hormone was acceptable during thyroxine treatment. Because thyroxine is so frequently prescribed to treat hypothyroidism, suppress thyroid nodules, and treat cancer and nontoxic goiters, its role in the development of osteoporosis appears to deserve renewed consideration.

Overproduction of male hormones may cause hirsutism—excess facial and body hair—in women, as illustrated in this 1877 drawing. Researchers at the University of Pittsburgh School of Medicine and the GCRC at Children's Hospital in Pittsburgh, Pennsylvania, are using hormone stimulation tests as diagnostic tools to differentiate causes of hirsutism.

Journal of Mental Science 23:86, 1877-1878

A young patient at the University of Florida GCRC in Gainesville shows Dr. Noel K. Maclaren a new T-shirt. Dr. Maclaren and his coworkers have found that immunosuppressive treatment may prevent or delay progression of diabetes in newly diagnosed patients with diabetes.

Patrick Dyson, University of Florida Health Science Center

Evidence for Amyloid Gene Defect in a Type of Alzheimer's Disease. According to investigators at the University of Indiana GCRC in Indianapolis, a mutation in a gene for amyloid precursor protein has been found in two recent generations of a family with familial, early onset Alzheimer's disease. Analysis of autosomal DNA from living and deceased family members revealed a specific mutation believed to be located in a DNA region that codes for amyloid protein. Direct sequencing of DNA from a family member with autopsy-proved Alzheimer's disease led to the identification of a single amino acid substitution—phenylalanine for valine—in the relevant area of the amyloid precursor protein. The mutation correlated with the presence of Alzheimer's disease in all patients studied and was absent in disease-free family members and 100 unrelated individuals. This study suggests that the mutation may be the inherited factor causing amyloid fibril formation and dementia in individuals with this form of Alzheimer's disease.

Diabetes

Glycosylation Endproducts Associated With Diabetic Complications. A team of scientists at Rockefeller University's GCRC in New York City reported during the year that the excessive accumulation of glucose-modified proteins and peptides, called advanced glycosylation endproducts (AGE's), appears to be involved in the chronic complications of diabetes.

The scientists measured circulating and tissue AGE levels in diabetic and nondiabetic patients who had varying degrees of kidney failure. They found the AGE content of specimens taken from the arterial walls of diabetic patients with endstage kidney disease to be almost twice as high as those from diabetic patients without kidney disease. All of the diabetic patients' arterial wall specimens had significantly higher levels of AGE's than did the specimens from nondiabetic normal controls. A patient who had normal kidney function after kidney transplantation had a blood level of AGE-peptides similar to persons with diabetes but without endstage kidney disease. The concentration of AGE-peptides circulating in the blood of diabetic patients correlated with the severity of their kidney dysfunction.

Could Insulin Aerosols Replace Insulin Injections? In a small-scale evaluation at the outpatient GCRC of Johns Hopkins University Hospital in Baltimore, Maryland, four patients with non-insulin-dependent diabetes mellitus and two nondiabetic persons received aerosolized insulin by inhalation through the mouth.

The Baltimore scientists hypothesized that earlier poor responses were due to an inadequate dose of insulin, probably as a result of drug loss before its entry into the blood supply. They therefore directed their efforts to developing a system that could deliver a maximal deposition of insulin in the lungs.

The investigators reported that blood samples collected from the participants after inhalation showed that a maximal decrease in their blood glucose concentration occurred from 40 to 200 minutes after insulin inhalation. The blood glucose level was significantly related to the dose of insulin deposited in the lungs; no adverse side effects were reported.

The investigators note that it is not known how long aerosolized insulin can maintain a lower blood glucose concentration, or whether the aerosolized form of insulin will be effective in obese patients. In addition, they need to evaluate the effectiveness of the new insulin delivery system after patients ingest a meal with known glycemic potential.

Studies to answer these and other questions are continuing in the outpatient GCRC at Johns Hopkins.

Heart Disease and High Blood Pressure

Monocyte-Activated Cytokines in Chronic Heart Disease. Investigators at Mount Sinai Hospital GCRC in New York City continued their studies of patients with chronic heart failure in efforts to learn the origins of the cachexia, or wasting, that affects these patients. Earlier they had found that high levels of a cytokine, or cell secretion, called tumor necrosis factor (TNF) were associated with heart cachexia and anemia, both classic markers of chronic heart failure. The source of the increased TNF, however, was not known.

Recently the Mount Sinai scientists studied levels of another cytokine, neopterin, which is produced with TNF when blood cells of the monocyte type are challenged by an immunologic stimulus. They measured neopterin levels in 52 patients with chronic heart failure and found the average level to be significantly higher than the neopterin level in control patients.

Chronic heart failure patients with both high neopterin and low neopterin levels shared neurohormonal and other variables but differed in one important outcome. High-neopterin patients had a lower 1-year survival than did those patients who had low neopterin levels in the blood. Moreover, patients with high neopterin levels weighed less

Molecular virologist Dr. Mary Estes and her colleagues at Baylor College of Medicine in Houston, Texas, cloned Norwalk virus, a single-stranded RNA virus, after purifying it from stool. The Norwalk virus is the major cause of viral gastroenteritis epidemics worldwide.

Bharat Parikh, Baylor College of Medicine

and had a greater degree of malnutrition than did low-neopterin patients. Low hemoglobin levels, which indicated the extent of anemia, and lower total lymphocyte counts were also similar to the clinical picture of heart failure patients with elevated TNF seen in the earlier study.

The Mount Sinai scientists have concluded that activated monocyte secretion of cytokines occurs in all heart failure patients and is the most likely source of the elevated levels of circulating neopterin and TNF that were found in these patients.

Reducing Arterial Buildup of Fats in Coronary Heart Disease. Investigators at the GCRC of the University of Washington in Seattle evaluated the effectiveness of various drug regimens in reducing coronary atherosclerosis, a condition in which blood vessels to the heart become narrowed by fatty buildup. The condition often leads to heart attack.

For a study nicknamed FATS—familial atherosclerosis treatment study—the investigators selected 145 men who were under 63 years old. The men had family histories of premature heart disease, elevated levels of a type of cholesterol associated with increased risk of heart disease, and evidence of heart disease. The men were randomly assigned to one of three regimens. One regimen was considered conventional therapy; the other two were regarded as aggressive treatment and involved administration of the cholesterol-lowering drugs lovastatin, colestipol, and niacin.

After 2.5 years 120 of the volunteers completed the program. Arteriograms to assess the fatty buildup in their coronary arteries were taken at the GCRC for comparison with those taken at the start of the study.

The Seattle scientists found that the conventional treatment was relatively ineffective, whereas both of the aggressive regimens lowered cholesterol levels and opened blocked arteries. The investigators concluded that the likelihood of progressive atherosclerotic disease was cut in half by aggressive therapy.

Calcium as Well as Salt in Diet Affects Blood Pressure. Endocrinologists at the GCRC of Brigham and Women's Hospital in Boston, Massachusetts, are concluding that salt does not act alone in elevating blood pressure in salt-sensitive hypertensive patients. Their studies, conducted first in Oriental and black patients, and now primarily among Caucasians with high blood pressure, demonstrate that high-sodium diets, coupled with a low calcium intake, interact to cause a rise in blood pressure. The Boston scientists measured blood pressure and kidney blood flow in a 2-week dietary study of 19 Caucasian and 2 black hypertensive patients.

During the first week all patients consumed a high-salt diet coupled with either low or high calcium intake. During the second week all patients consumed a low-salt, low-calcium diet.

At the end of the first week blood pressure had increased in all patients. However, patients receiving the high-salt, low-calcium diet had a significantly greater increase in blood pressure than did those receiving the diet high in both salt and calcium.

The investigators have reported that low calcium intake also has an adverse effect on renal blood flow. They suggest that low-calcium diets may contribute to the overall phenomenon of salt sensitivity.

Maternal and Infant Health

Calf-Derived Surfactant Benefits Premature Infants' Lungs. During 1991 investigators at the University of Rochester School of Medicine GCRC in New York reported on the use of a calf-derived surfactant to assist respiration in premature infants at risk of a life-threatening neonatal disorder called hyaline membrane disease. In a randomized clinical study of 479 babies born prematurely at three New York medical centers, they found that 88 percent of the infants treated at delivery with prophylactic doses of the surfactant—which helps the lungs expand smoothly during breathing—survived and were later discharged to go home without ventilatory assistance.

Other premature babies who received surfactant as a "rescue" procedure after delivery also benefited. However, the survival advantage of receiving surfactant therapy immediately at birth, or prophylactically, was particularly striking in a subgroup of premature infants whose gestational age was estimated to be 26 weeks or less.

Seventy-five percent of those very premature infants receiving prophylaxis—in contrast to 54 percent of "rescue therapy" recipients—survived. Pneumothorax, an accumulation of air in the pleural space surrounding the lungs that often leads to total lung collapse and death, occurred in 7 percent of the very premature infants in the prophylaxis group and in 18 percent of those receiving surfactant as a "rescue therapy."

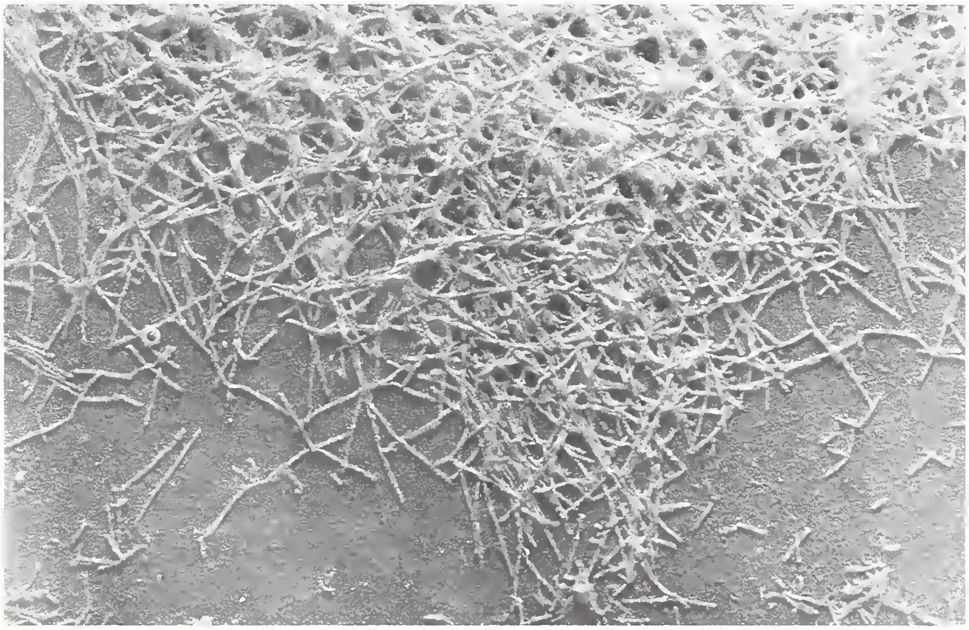
Maternal-Neonatal Infection by Herpes Simplex Virus-2. Scientists at Stanford University's GCRC in Stanford, California, studied 1,891 pregnant women of predominantly low socioeconomic status throughout their pregnancies to determine if the mothers had evidence of infection by herpes simplex virus type 2 (HSV-2), which primarily affects the genital areas. Each year approximately 1,600 babies are born with herpes in the United States.

An immunochemical test showed that 311 women, more than 16 percent, had evidence of a past infection in both early- and late-gestation blood samples. Four women who tested HSV-2 negative early in the study developed HSV-2 antibodies later in the pregnancy. The infants of these four mothers were delivered vaginally at term, and no complications were observed during the neonatal period. The babies' heights, weights, and head circumferences were normal; no abnormalities were seen during physical examination. The investigators have concluded that pregnant women can acquire an initial asymptomatic HSV-2 infection; moreover, such asymptomatic primary episodes of HSV-2 may not be damaging to their offspring.

The same group at Stanford developed a sensitive new test for the presence of HSV-2 in clinical samples. The new test, involving a polymerase chain reaction, is able to detect very small quantities of the virus in specimens from asymptomatic pregnant women with genital HSV infections.

Mental Disorders

Alzheimer's Disease Progression Is Delayed by Acetyl-L-Carnitine. A double-blind, controlled clinical evaluation of acetyl-L-carnitine as a treatment of Alzheimer's disease was con-



Fine fibers of amyloid protein accumulate in organs and tissues of patients with amyloidosis, a group of often fatal disorders studied in the GCRC at Boston University School of Medicine in Massachusetts. Amyloid is also found in the brain of patients with Alzheimer's disease, which affects an estimated 10 percent of the elderly in the United States.

Courtesy of Dr. A. S. Cohen, Boston University School of Medicine ducted in the ambulatory and inpatient units of Columbia University's GCRC in New York City. The dementia caused by Alzheimer's disease affects as many as 10 percent of Americans over age 65. Findings in 30 patients indicate that the drug may slow the progression of Alzheimer's disease.

When task-handling competence was evaluated after 6 months of treatment, more patients receiving placebo had declined in competence than had those receiving acetyl-L-carnitine, the investigators found. Patients receiving the test drug improved in their ability to handle tasks requiring attention and concentration. Baseline cognitive scores improved more dramatically in a subgroup of patients with the most severe dementia than in those who functioned at a higher level prior to the study. Moreover, followup measurements of cerebrospinal fluid (CSF) showed greater CSF concentration of acetyl-L-carnitine in patients in this subgroup than in those who showed a less impressive change in mental function.

The study provides direct evidence that acetyl-L-carnitine enters the brain and may help patients with relatively advanced disease.

Dementia Associated With Stroke and Parkinsonism. When researchers at Columbia University GCRC in New York City examined 226 elderly patients hospitalized for stroke 3 months earlier, they found dementia in 15 percent of the patients. In these elderly persons race was correlated strongly with dementia; the incidence of dementia was seven times greater in nonwhites than in whites. Earlier strokes and diabetes also increased the risk of dementia.

The investigators found that dementia was prevalent among patients whose stroke had occurred in the left hemisphere of the brain. Gender and educational status were not factors in the prevalence of dementia in these stroke patients, nor was a previous history of high blood pressure or heart disease.

The same group of researchers studied patients with Parkinson's disease living in a northern New York City area. The investigators found that the overall prevalence of dementia was much higher than expected—29 percent in the persons with Parkinson's disease versus 4 percent in a community comparison group. Dementia prevalence increased with increasing age, affecting 14 percent of persons aged 60 to 69 and nearly 60 percent aged 80 to 89; the condition was more prevalent in persons with fewer than 8 years of formal education. The Columbia research team believes that earlier low estimates of dementia with parkinsonism may have been biased because of case selection or methods of diagnosis. They conclude that the actual prevalence of dementia in patients with Parkinson's disease is at least twice that previously reported.

Obesity

Individual Oxidation Patterns Adjust to Diet but Fats Remain a Problem. Increasing the fat or carbohydrate content of a diet triggered individuals in a nutritional study at Vanderbilt University in Nashville, Tennessee, to rapidly increase their rates of oxidation (calorie burning) of fats or carbohydrates to correspond with increasing intake.

The investigators employed a room-size calorimeter to assess the effects of dietary composition on calorie burning versus calorie storage. They found that when food intake was fixed for 7 days at levels near a person's caloric requirements, changes in the fat/carbohydrate ratio in the diet produced rapid (within 3 days) adjustments in a person's rate of oxidation of fat and carbohydrate. High-fat diets rapidly increased fat oxidation and high-carbohydrate diets rapidly increased carbohydrate oxidation. In a followup study the scientists evaluated the volunteers' responses to changes in dietary composition when total food intake was not fixed. For 1 week lean and obese volunteers ate a high-fat diet and during a second week ate a diet high in carbohydrates. During the first week all volunteers increased their carbohydrate oxidation so that on day 7 it matched or nearly matched the increase in carbohydrate intake.

During the week of high-fat feeding, however, fat oxidation increased significantly in the lean subjects but was nevertheless much less than the fat intake. In the obese subjects fat oxidation was not significantly increased.

The Vanderbilt scientists conclude that an excess intake of protein or carbohydrate is not as critical as excessive dietary fat in the development of obesity.

Biomedical Research Technology Program

Program Description

The Biomedical Research Technology Program (BRTP) supports and promotes the development and use of leading-edge technologies by the biomedical research community. By funding 58 resource centers across the country the BRTP provides scientists with access to expensive state-of-the-art equipment and also with the necessary training to utilize these sophisticated instruments to their full potential. The current emphasis of the program is on extending the biomedical applications of computers, engineering new instruments to solve biomedical problems, and advancing the study of molecular and cellular structure and function through application of specialized technologies.

In FY 1991 the NCRR provided about $40.9 million for the BRT Program, approximately $33 million of which was spent in support of the resource centers. Each center functions as a national or regional resource for the biomedical research community and offers advanced equipment and technologies that are among the best in the world. Staff scientists at the resource centers collaborate with outside biomedical investigators to solve research problems using the technologies of the resource. Among the available instruments are high- and intermediate-voltage electron microscopes; advanced laser instrumentation; mass spectrometers; magnetic resonance spectrometers; x-ray diffraction and detection instruments; and a variety of computer systems for modeling molecular interactions and disease progression, image analysis, and supercomputing.

In addition to providing funds for resource centers, the BRTP also awards several other types of grants. Approximately 15 Small Grants for Innovative Technology are awarded each year in support of small-scale projects to stimulate the development of new and unproved equipment and procedures. The BRTP also supports an active Small Business Innovation Research Grants Program, which provides funds to small businesses for research into new technologies, instrument development, and improvements to existing technologies.

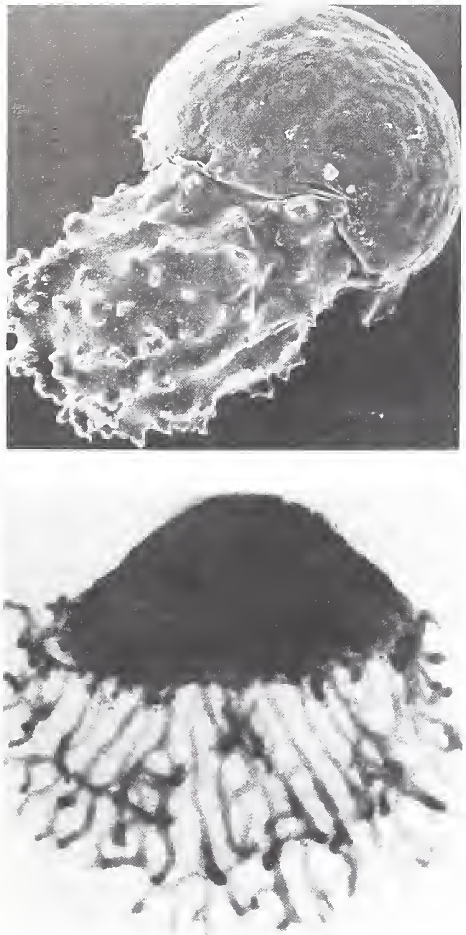
Other types of funding available from the BRTP include Pre-Resource Research Grants, which are used to develop and assess the feasibility of new technologies for biomedical research; First Independent Research Support and Transition (FIRST) Grants, which provide initial support to newly independent biomedical investigators; and Investigator-Initiated Research Grants, which support research and development of new instruments or technologies that may have broad application to biomedical research. The BRTP is also authorized to award conference grants, Academic Research Enhancement Awards, cooperative agreements, and contracts to carry out its mission.

Communication with the biomedical research community is an important component of the BRTP's objectives. Through publications, seminars, workshops, and conferences BRTP grantees disseminate research achievements and announce training opportunities and the availability of technical information in the resource centers.

Research Highlights

Advances in Structural Biology Research. Structural biology comprises structural studies of biological molecules at atomic and near-atomic resolutions and studies of processes such as viral assembly and disassembly, transport of molecules across cellular membranes, and the immune response. Recognizing the need for multidisciplinary cooperation in this rapidly developing area, the BRTP sponsored a 2-day workshop that brought together experts in the fields of biology, chemistry, biophysics, medicine, and computer science. Titled "Technologies for the Future: Opportunities and Needs in Structural Biology and Molecular Medicine," the workshop reviewed recent achievements and charted a course for future research efforts. In the summer of 1991 the BRTP released a 40-page planning document that highlighted the key points and recommendations made by workshop participants.

Since the 1950's, when the three-dimensional crystal structures of hemoglobin and myoglobin were first determined through scrutiny of x-ray diffraction patterns, enormous strides have been made in the study of biological molecules and processes. X-ray crystal-



Sperm cells of the pig intestinal worm Ascaris suum *have an unusual system of locomotion, which was elucidated at the University of Wisconsin-Madison Integrated Microscopy Resource. Lacking flagella, the whip-like projections that propel most other types of sperm, the* Ascaris *sperm uses a pseudopod, or bag-like extrusion, to crawl along the female reproductive tract. By removing the pseudopod membrane, scientists uncovered an elaborate cytoskeleton that assembles and disassembles constantly during movement. A scanning electron micrograph (top) shows an intact pseudopod beneath the rounded sperm cell body; a stereo-electron micrograph (bottom) shows the exposed cytoskeleton of the pseudopod.*

Courtesy of Dr. S. Sepsenwol and Journal of Cell Biology

lography is still the most widely used and reliable method for determining the structures of proteins and related compounds, but advances in x-ray absorption spectroscopy, nuclear magnetic resonance (NMR) techniques, and other methods also allow the study of molecules that do not crystallize or are not suitable for x-ray diffraction experiments for other reasons. In addition, high-performance computers and graphics programs can analyze enormous quantities of data and provide additional insights into structure-function relationships.

With BRTP support, these technologies are being developed and made available to the scientific community. Armed with a deeper understanding of structural biology, researchers are gaining important insights into the molecular mechanisms of disease and are designing new drugs to block specific molecules that contribute to the progression of a disease.

Synchrotron Resources for Biomedical Research. When electrons circle through the particle raceway of a synchrotron, intense multiple-wavelength radiation is produced. The radiation can be harnessed and used in x-ray diffraction experiments, x-ray scattering, x-ray absorption spectroscopy, and other methods for determining biological structure and function. Synchrotron radiation is ideal for such studies because its high intensity allows the generation of high-resolution data that can be collected more quickly than is possible with other x-ray sources. Shorter exposure time also causes less sample degradation by the X rays. The BRTP funds synchrotron resources for biomedical studies at three national laboratories: the Cornell High Energy Synchrotron Source, the Stanford Synchrotron Radiation Laboratory, and the Brookhaven National Laboratory.

Using a beamline from the National Synchrotron Light Source at Brookhaven, scientists at the BRTP-supported CCD Area Detector Research Resource are developing new and powerful x-ray detectors based upon charge-coupled devices (CCD), which allow direct quantitative readings of the intensities of diffracted X rays. The resource's new detectors were recently employed in determining the three-dimensional structure of the cholera toxin. Each year cholera afflicts hundreds of thousands of people worldwide.

A protein produced by the *Vibrio cholerae* bacterium, the toxin causes severe diarrhea in cholera patients, who may die from dehydration. After taking more than 25,000 x-ray diffraction measurements of the toxin crystal, the scientists used computer analysis to produce an image of the three-dimensional structure. The toxin appears to consist of a large doughnut-shaped protein subunit attached to a much smaller subunit.

The investigators speculate that the larger subunit binds to the outside of intestinal cells, while the other subunit slips inside and initiates a series of adverse reactions. As a result, the body loses copious amounts of water through the intestinal cells, causing diarrhea. Understanding the toxin's structure may aid in the development of improved vaccines or drug therapies.

X-ray Diffraction Studies Reveal Protein Kinase Structure. The BRTP supports several nonsynchrotron resources that use X rays to determine biological structure and function. Working at the BRTP-supported Resource for Protein Crystallography at the University of California, San Diego, scientists made a major breakthrough in determining the three-dimensional structure of the protein kinase enzyme that plays key roles in the pathogenesis of cholera, cancer, high blood pressure, and other diseases.

The protein kinases, a family of more than 100 enzymes, are crucial to the metabolism and growth of eukaryotic, or nucleated, cells. Although the sizes and functions of the various eukaryotic protein kinases differ, each possesses an identical catalytic core that binds to and activates other proteins of the cell.

At the Resource for Protein Crystallography a sophisticated system of detectors measured diffracted x-ray intensities from protein kinase crystals; analysis of these data with the use of advanced computer graphics provided detailed structural information about the catalytic core and the core bound to a peptide inhibitor. The catalytic region of the protein kinase was found to have two lobes. One binds to the energy-rich molecule adenosine triphosphate (ATP); the other binds to a protein that becomes activated by accepting a high-energy phosphate group from ATP.

These findings may have broad implications for understanding the biological mechanisms employed by all protein kinases in eukaryotic cells, including human cells. The three-dimensional atomic coordinates for the bound and the unbound catalytic cores have been deposited in the Brookhaven Protein Data Bank, which is partially funded by BRTP.

Nonradioactive Markers of Biological Processes and Structures. Stable isotopes, which occur in tiny amounts in nature, provide unique noninvasive probes for the study of biological structures and processes. The BRTP-funded National Stable Isotopes Resource (SIR) at Los Alamos National Laboratory in New Mexico supplies investigators with nonradioactive biological molecules tagged with these rare forms of carbon, nitrogen, oxygen, or sulfur.

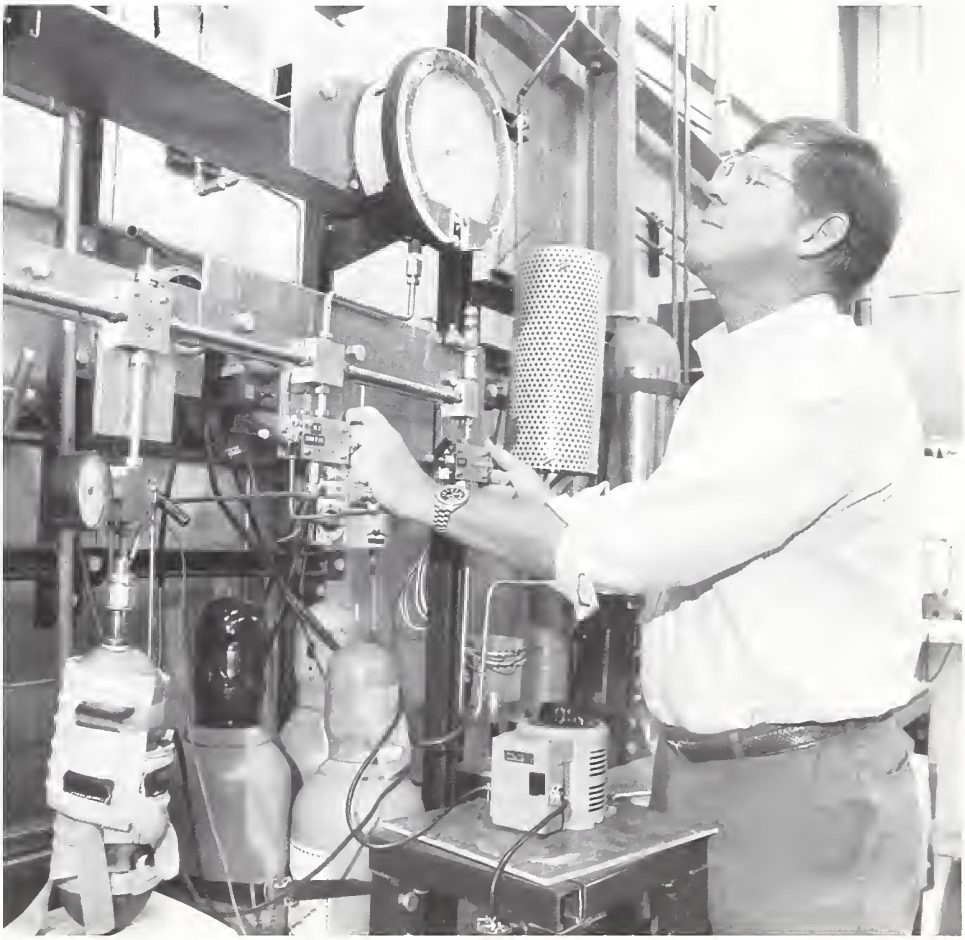
When stable isotopes are incorporated into specific sites in proteins or nucleic acids, researchers can obtain detailed information about the structure, dynamics, and function of these macromolecules by using nuclear magnetic resonance (NMR) spectroscopy, mass spectrometry, or other techniques. Because stable isotopes are not radioactive, they can be used safely as biological markers in clinical research and are often employed in metabolic studies in children or pregnant women.

To study a group of enzymes known as serine proteases, investigators used nitrogen-15 provided by the SIR to label an amino acid that is found in the binding sites of these enzymes. Serine proteases are crucial to a host of complex physiological processes, including blood clotting, immune responses, and cellular differentiation. In a series of experiments that employed NMR techniques, the investigators were able to resolve earlier inconsistent interpretations of the protease structure. Armed with a deeper understanding of the enzymes' binding sites, the scientists are now considering the role serine proteases may play in T-lymphocyte activation and immune regulation.

Viral Assembly Process Studied by NMR Analysis. Advances in NMR spectroscopy allow scientists to explore the finer details of noncrystallized molecular structures that previously were beyond their ken. Using stronger magnetic fields and more powerful computational methods, researchers can determine the structures and functions of proteins in solution. The techniques of solid-state NMR allow structural analysis of immobile proteins such as those embedded in cellular membranes.

In a concerted approach that combines these powerful new NMR methods, University of Pennsylvania researchers used the facilities at the BRTP-supported National Magnetic Resonance Facility at Madison, Wisconsin, to uncover the most detailed structural information yet available for modeling the assembly of a viral coat at the cellular membrane. The scientists' findings may have broad implications for understanding other membrane-mediated processes of the cell, including protein secretion and assembly of membrane components.

When rod-shaped viruses known as filamentous phages infect bacteria, they disassemble as they enter the host cell and distribute their coat proteins throughout the bacterial membrane. The viral DNA is released into the cell. When new viral DNA has been pro-



Dr. James A. Fee monitors the synthesis of ^{13}C-labeled methanol at the Los Alamos Stable Isotope Resource (SIR) in New Mexico. Simple molecules like methanol, ammonia, and water are tagged with stable nonradioactive isotopes and then incorporated into biologically important compounds like amino acids, carbohydrates, or lipids that are used for research.

Dr. James A. Fee, Los Alamos

A computer-aided laser microsurgery system produced with BRTP funding recently made the move from laboratory bench to operating room. Developed at the Massachusetts Institute of Technology in Cambridge, the system is being used in clinical trials at the Cleveland Clinic in Ohio to unblock coronary arteries. A catheter carries laser light to an arterial plaque, and a computer screen reveals which areas of the catheter tip are touching diseased tissue. High-energy laser light is fired down selected fibers to remove the blockage while leaving healthy tissue intact.

Cleveland Clinic Foundation

duced and is ready to be released in viral particles, the coat proteins are recycled and encase the viral DNA as it is secreted from the cell.

To uncover the details of the viral assembly process investigators performed two-dimensional NMR analysis on coat proteins labeled with stable isotopes. This technique delineated the molecule's secondary structure, or the spatial characteristics of the protein chain. In further experiments the scientists used solid-state NMR to determine the three-dimensional structure, or folding, of membrane-bound proteins and neutron diffraction studies to visualize the shapes of coat proteins in the intact virus.

The three-dimensional structure of each coat protein was found to undergo a remarkable transformation as it left the host cell membrane and linked to other proteins in surrounding the secreted DNA. The protein's secondary structure, however, remained essentially unchanged. The investigators suggest that these types of changes may be common characteristics of proteins as they are inserted into or transported across cellular membranes.

Supercomputers Simulate the Dynamics of Large Molecules in Solution. By applying the high-speed analysis and large memory capacity of supercomputers to biomedical research, investigators are now tackling difficult problems once considered infeasible. The BRTP supports major new developments in this fast-moving field through funding several resources for biomedical computing.

At the Pittsburgh Supercomputing Center scientists from Wesleyan University in Middletown, Connecticut, and the Bristol-Myers Squibb pharmaceutical company in Willingford, Connecticut, used the Cray Y-MP supercomputer to simulate the dynamic movements in solution of the enzyme HIV protease, which is essential to replication of the AIDS virus. A target of considerable study, the three-dimensional structure of the HIV protease has been well-characterized by x-ray crystallography, but in the watery environment of the cell, hydrogen bonding and electrostatic forces can dramatically alter the shapes and movements of any large biological molecule, including the protease.

Details of the protease's crystal structure were obtained from the BRTP-supported Protein Data Bank at Brookhaven National Laboratory in Upton, New York. The scientists then employed the Cray supercomputer, which simulated the motions of the nearly 200 amino acids that make up the enzyme and of 7,000 surrounding water molecules, as the protein's three-dimensional shape evolved at picosecond (trillionth of a second) time scales.

The researchers observed a flexible molecular "flap" near the substrate binding site of the enzyme and detected a molecular "cantilever" that apparently moves up as the flap moves down. Never before identified, the cantilever may be crucial to the enzyme's normal function and thus may be an important new target for inhibiting protease action and developing AIDS virus inhibitors.

Comparative Medicine Program

Program Description

The significant contributions of laboratory animals to biomedical research are well known. Almost every advance in the prevention and treatment of important human diseases during the past century has involved the use of experimental animals. The Comparative Medicine Program (CMP) continues to support a large number of projects related to research animal resources that are essential for investigations of human diseases. The three major areas of the CMP are the Regional Primate Research Centers Program, the Laboratory Animal Sciences Program, and the AIDS Animal Models Program.

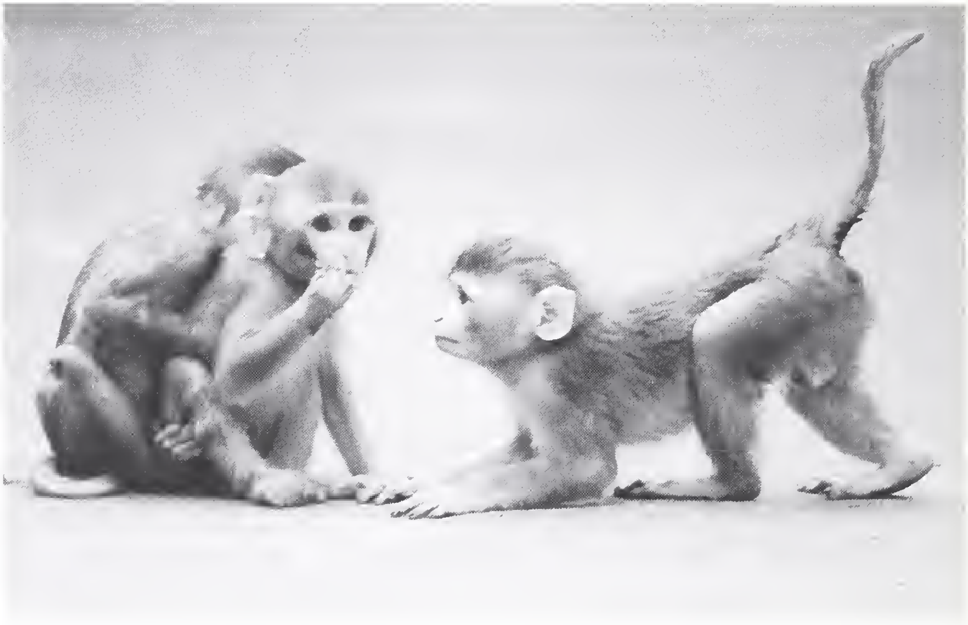
During FY 1991 the CMP received a total allocation of $68.5 million to provide core support for the seven Regional Primate Research Centers; to upgrade laboratory animal facilities at biomedical research institutions; to provide diagnostic and laboratory services for colonies of research animals; to train professional personnel to study and care for laboratory animals; to identify and develop new and improved animal models for studies on human diseases; to breed certain scarce species, thereby increasing the reserves of valuable research animals; to promote the conservation of these species; and to distribute knowledge related to the optimal care and use of laboratory animals.

The Regional Primate Research Centers were funded at a level of $37.4 million in FY 1991. A major benefit of this support of research in primatology is the identification of important animal models for human diseases. The seven centers continue to identify and develop valuable models for studies on many human diseases including hypertension, thrombosis, ulcerative colitis, cancer of the colon, leprosy, herpesvirus infection, Parkinson's disease, and AIDS. Through careful studies on naturally occurring diseases of nonhuman primates and concentrated efforts to understand a condition comparable to AIDS in humans, the Primate Centers identified a simian immunodeficiency virus (SIV). The SIV is very similar to the human immunodeficiency virus (HIV), which causes AIDS in humans, and was demonstrated to cause an AIDS-like disease in nonhuman primates. The SIV macaque model is now widely recognized as the most important animal model for studies on AIDS. This unique model would probably not have been discovered without the concerted and intense efforts within the Regional Primate Research Centers Program. Other models of important human diseases await discovery and development in nonhuman primates. These include models for Alzheimer's disease, infant mortality, reproductive failure, and therapeutic gene transfers.

Approximately $4.5 million was awarded to continue the Chimpanzee Breeding and Research Program that was initiated in September 1986. Chimpanzees are excellent models for investigations of certain human viral diseases, and their use contributed greatly to the successful development of an effective and safe vaccine against hepatitis B. The chimpanzee is the only animal that can be infected successfully with HIV. For this reason scientists continue to believe that the chimpanzee will be critical to the development of an effective and safe human AIDS vaccine. This breeding program has been extremely successful to date, producing 225 offspring from September 1986 to July 1991.

Approximately $2.0 million was awarded to continue the Specific-Pathogen-Free (SPF) Rhesus Breeding and Research Program, which was initiated in 1988 to provide monkeys free of certain retroviruses and herpes B virus. The goal is to produce at least 500 SPF rhesus monkey offspring each year for Public Health Service-supported AIDS-related research and other investigations requiring the use of retrovirus-free animals. Six breeding projects and two related research projects (behavioral and genetic) are supported in this effort. The importance of this breeding program for biomedical research is underscored by recent events related to the de facto embargo on importing several species of nonhuman primates used in biomedical research.

High-quality housing and care must be provided to ensure the health and well-being of laboratory animals. In FY 1991 approximately $11.0 million was awarded to upgrade animal facilities and care at institutions throughout the United States. Sanitation equipment, modern caging and runs, and efficient heating/ventilation/air conditioning (HVAC) sys



Young rhesus monkeys explore their surroundings at the Oregon Regional Primate Research Center, located on a 12-acre tract in Beaverton and associated with the Medical Research Foundation of Oregon, Beaverton.

Harry Wohlstein, Oregon Regional Primate Research Center

tems were provided through CMP support to improve environmental quality in these facilities.

Postdoctoral training for graduate veterinarians in the field of laboratory animal medicine continues to be emphasized because of the significant need for specialists in this field. Seventeen institutional programs were supported by the CMP under the National Research Service Award program. These institutional programs provided specialized training for 77 individuals to ensure high-quality management and medical care for laboratory animals. In addition, five individual postdoctoral research training awards were made. Eleven Special Emphasis Research Career Awards were also granted to graduate veterinarians who are committed to biomedical research careers.

Regional Primate Research Centers Program

Since their establishment in the early 1960's, the seven Regional Primate Research Centers have served the nation's biomedical research needs in a unique and essential fashion. Nonhuman primates are very often the vital link between basic research and human health applications. In some cases only a study of nonhuman primates will provide answers to particular medical or behavioral questions. Through these seven centers the NIH has provided a cost-effective response to the need for national repositories of primates, scientific expertise, and the specialized facilities and equipment necessary for biomedical research on primates.

The seven centers and the host institutions with which they have close administrative and research relationships are:

- **California Regional Primate Research Center,** Davis, associated with the University of California at Davis;
- **Tulane Regional Primate Research Center,** Covington, Louisiana, associated with Tulane University, New Orleans;
- **New England Regional Primate Research Center,** Southborough, Massachusetts, associated with Harvard University, Cambridge;
- **Oregon Regional Primate Research Center,** Beaverton, associated with the Medical Research Foundation of Oregon, Beaverton;
- **Washington Regional Primate Research Center,** Seattle, associated with the University of Washington, Seattle;
- **Wisconsin Regional Primate Research Center,** Madison, associated with the University of Wisconsin, Madison;
- **Yerkes Regional Primate Research Center,** Atlanta, Georgia, associated with Emory University, Atlanta.

The centers provide the basic elements necessary for more than 1,200 American scientists to conduct primate research. The scientific missions of these investigators serve all of the categorical institutes of the NIH as well as other governmental, charitable, and commercial agencies that support biomedical research. These scientists represent 383 research institutions. In addition to serving investigators on-site, the centers' tissue distribution programs provide more than 10,000 specimens per year to laboratories throughout the country. Overall the centers provide access to over 16,000 nonhuman primates, representing 32 species that have been found valuable for basic biological and medical research. Many of the animals are in breeding colonies that are essential to our nation's biomedical research needs and represent an irreplaceable national resource.

Nonhuman primates share about 90 percent of the human genome and so exhibit many characteristics that make them uniquely valuable for research pertinent to human health. The expanded primate forebrain, human-like visual and auditory systems, and complex social and communicative behavior allow basic neurobehavioral research into human capacities and mental disorders that no other research animals provide. Their cholesterol metabolism, mechanisms of fat deposition, and physiological responses to emotion make them essential for cardiovascular research. As the only animals with a menstrual cycle comparable to that of human females, nonhuman primates provide essential animal models for analyzing critical aspects of female reproductive biology and pathology. Their susceptibility to diseases such as poliomyelitis, measles, and AIDS that also affect humans has made them the species of choice, and in some cases the only species, for studying those diseases. The susceptibility of prematurely born nonhuman primates to syndromes comparable to those seen in premature human infants provides the best model for developing therapies for the many complications of prematurity. Finally, the longevity of nonhuman primates, approximately 10 times that of laboratory rodents, makes them excellent models for the aging process in the longest-lived mammalian species, the human.

AIDS Animal Models Program

The AIDS Animal Models Program supports research and resources that identify, develop, and make available animal models for AIDS-related investigations. The program promotes the characterization and validation of existing models and coordinates these efforts with other NIH institutes.

Basic and clinical research on AIDS-related disease has been greatly accelerated by the development of nonhuman primate models that are susceptible to HIV infection (chimpanzee) and SIV infection (macaque) and manifest, both serologically and clinically, the symptoms observed in human AIDS.

Chimpanzee Breeding and Research Program. The Chimpanzee Breeding and Research Program (CBRP) is the first and only national program to converge the expertise and experience of chimpanzee breeders and researchers for a combined effort. This multi-institutional program, now in its fifth year of operation, has been exceedingly successful in providing the Public Health Service-supported biomedical research community with an adequate supply of chimpanzees for research on important human health problems, including viral hepatitis and AIDS.

The Comparative Medicine Program administers the chimpanzee resource, and the CBRP Management Committee provides oversight and advice. The committee develops

Studies using rabbits and rhesus monkeys have shown that administration of a monoclonal antibody, known as MAb 60.3, protects animals from generalized organ damage in connection with severe bleeding and shock. If the effect can be confirmed in humans, in the future MAb 60.3 might be administered to severely injured people. Dr. Nicholas B. Vedder, of the University of Washington School of Medicine, who collaborated with investigators at the Washington Regional Primate Research Center in Seattle, is examining a rabbit that participated in the study.

Mary Levin, University of Washington

policies related to a wide range of issues including use of chimpanzees in infectious disease research, safety considerations for personnel working with chimpanzees, and environmental enrichment for the captive population.

The CBRP is designed to provide a stable supply of healthy chimpanzees for biomedical research and to perpetuate the captive chimpanzee population in the United States. There are currently 475 disease-free adult breeding chimpanzees in the program's colonies, and a total of 225 offspring have been produced since the inception of the program in 1986. Forty-five percent of these offspring are mother-reared; the remainder are reared with other young chimpanzees in nursery-type environments. At present the CBRP animal population is growing at a rate of 5 percent a year, but by design the population will stabilize within the next 2 years to a maintenance growth rate.

Research projects integrated into the breeding program correct gaps in scientific knowledge and problems affecting the reproduction of chimpanzees in captive environments. The projects focus primarily on behavioral development, reproduction, genetics, health, demography, and breeding management. These studies are conducted at the University of Texas M.D. Anderson Science Park at Bastrop; the Primate Foundation of Arizona in Tempe; the University of Southwestern Louisiana's New Iberia Research Center; the Yerkes Regional Primate Research Center in Atlanta, Georgia; the Regional Primate Research Laboratory at Holloman Air Force Base, New Mexico; the International Species Information System in Apple Valley, Minnesota; and the Nonhuman Primate Genetics Typing Laboratory at Trinity University in San Antonio, Texas.

The CBRP companion research studies complement and advance the ultimate goals of the program. Studies on genealogy show a large genetic variation in the captive chimpanzee population; controlled breeding techniques are minimizing the risk of inbreeding.

Behavioral studies are also conducted as part of the national program. Studies are under way to identify behavioral and developmental factors that accurately predict the chimpanzee's sociosexual behavior. It is now possible to understand some of these factors and develop intervening strategies that promote normal development in all of the participating captive chimpanzee colonies.

One of the new research projects integrated into the breeding program is designed to document the value of behavioral management through a more formal and intensified animal training program. Professional animal trainers employ the latest positive reinforcement training techniques to ease working with chimpanzees in a captive setting and simultaneously improve the animals' well-being.

Behavioral scientists at the University of Texas at Bastrop and the Primate Foundation of Arizona in Tempe are conducting objective, quantitative evaluations of methods to improve the care and production of captive chimpanzees. These scientists have made important advances in enhancing the well-being of chimpanzees by developing and studying environmental enrichment techniques such as providing the animals with a variety of durable and destructible toys, giving them articles they can use as tools (like chimpanzees in the wild), showing them videotapes of other chimpanzees, and initiating intensive feeding programs. Mechanisms are also being analyzed to minimize the negative impact of single caging when chimpanzees serve as biomedical research subjects. These studies play an important role in developing a self-sustaining population of behaviorally competent and psychologically healthy captive chimpanzees.

Reproduction and fertility research represents an important extension of the program. Since larger breeding animals are difficult to handle and expensive to maintain, efficiency in reproductive performance is highly desirable. Studies of the physiological events of sperm maturation and reactions in the female chimpanzee are being conducted, and this new and useful information will be applied directly to the breeding colonies. The results may suggest alternatives to natural breeding, such as embryo and sperm freezing (banking), embryo transfer, and the production of multiple births (twinning).

Intense efforts are made to ensure that chimpanzees continue to be used in the most appropriate and humane manner when employed as a research model. Chimpanzees from the CBRP may be assigned to U.S. Public Health Service or other government-supported research projects, pending approval of the research protocols by the Interagency Animal Models Committee. This review is in addition to the initial evaluations of the individual protocols by the respective Institutional Animal Care and Use Committees and the funding agency's peer review system.

Specific-Pathogen-Free Rhesus Breeding and Research Program. SIV research in rhesus macaques is of critical importance to the concerted AIDS research effort. The Comparative Medicine Program's specific-pathogen-free (SPF) *Macaca mulatta* (rhesus) breeding colonies are currently in their fourth year of development. Characterization and demographic status of the SPF colonies have been achieved, and it is now possible to estimate the production rate over the next 5 to 6 years. Expert retrovirologists recognize that an adequate and dependable supply of high-quality SPF rhesus monkeys is absolutely essential for future AIDS-related investigations.

To ensure that SPF rhesus monkeys are available for AIDS research, breeding colonies have been established at the Texas Primate Center of Hazleton Research Products in Alice; the New England Regional Primate Research Center in Southborough, Massachusetts; the University of Texas M.D. Anderson Science Park at Bastrop; the Michigan Department of Public Health in Lansing; the Laboratory Animal Breeders Service in Yemassee, South Carolina; and the University of Miami School of Medicine in collaboration with Charles River Laboratories, Key Lois, Florida.

The combined SPF colonies currently have more than 1,300 animals; the breeding females represent 43 percent of the total population. To support the continuing development of the colonies, all female offspring will be returned to the colonies for breeding during the next 2 to 3 years. Data from the six participating institutions have been pooled, and production rates of yearlings for the next 5 years have recently been projected. Given a probable reproductive rate of 70 percent, the national program will produce over 200 SPF yearlings in 1994. Most of the colonies' young males and retired breeders are currently available for AIDS research, with priority given to projects sponsored by the United States Public Health Service.

The breeding facilities have been furnished with modern caging equipment, and innovative long-term holding areas for the colonies are in place. In addition to the quantitative production aspects of making SPF animals available for AIDS-related research, the SPF program promotes research projects that will enhance production of healthy, well-defined animals through development of modern diagnostic tests, improved housing and innovative husbandry practices, computerized record-keeping systems, and new techniques that permit in-depth genetic monitoring.

Companion research activities in virology, genetics, and behavioral enrichment play a major role in support of program goals. The virology reference laboratories conduct testing for prevention and control of herpes B virus and simian retrovirus infection, participate in basic viral research, and provide strategies for ongoing colony production. The laboratories have performed more than 8,000 tests to ensure the SPF status of the colony animals. The herpes B virus laboratory of the Southwest Foundation for Biomedical Research in San Antonio, Texas, provides the program with rapid virological and serological screening and identification of possible *Herpesvirus simiae B* infection.

The Simian Retrovirus Reference Laboratory at the University of California Regional Primate Research Center in Davis focuses on identification and control of simian retroviruses in captive nonhuman primate populations, with particular emphasis on the SPF breeding colonies.

The Primate Genetics Reference Laboratory at the California Regional Primate Research Center provides SPF colonies with genetic information to enhance breeding potential. Monitoring and quantifying genetic heterozygosity is of primary concern in the macaque colonies and is being documented to expand the genetic profiles of the colonies.

The cryopreservation laboratory at the CMP-funded Jackson Laboratory in Bar Harbor, Maine, maintains a bank of more than 750,000 embryos, representing 860 strains of mice. The embryos are frozen at the eight-cell stage shown. Many of the strains represent important animal models for human diseases.

Dr. L. Mobraaten, The Jackson Laboratory

Laboratory Animal Sciences Program

The Laboratory Animal Sciences Program (LASP) continues to focus on improving the well-being of laboratory animals, investigating the cause and spread of diseases, and developing effective control and preventive measures. Studies include the identification, characterization, and development of new and improved animal models for use in biomedical research. Postdoctoral training in laboratory animal medicine is also an important area of emphasis. Specifically, the LASP seeks to:

- Study the normal and abnormal anatomy, physiology, genetics, and behavior of laboratory animals that are essential for biomedical research;
- Advance techniques needed to develop research-related animal resources. Of special interest is development of new, innovative techniques and procedures to create, produce, maintain, and preserve genetically designed transgenic animals and/or cells;
- Identify, characterize, and develop new and improved, naturally occurring and specifically targeted transgenic animal models for studying human diseases and developing genetic therapies;
- Investigate and study the etiology, diagnosis, pathogenesis, control, and prevention of laboratory animal diseases that adversely affect biomedical research;
- Develop new and more specific, sensitive, and rapid diagnostic techniques and procedures to detect and monitor infectious, genetic, and other diseases in lab animals;
- Train graduate veterinarians in the specialty of laboratory animal medicine and

In CMP-funded research to overcome a rare immunodeficiency disorder, Dr. Kateri A. Moore and her colleagues at Baylor College of Medicine in Houston, Texas, transferred a human gene for the enzyme adenosine deaminase (ADA) into mouse cells. The cells, which were transplanted into mice, subsequently produced the human enzyme that is lacking in children with congenital ADA-deficiency.

Bharat R. Parikh, Baylor College of Medicine

research in laboratory animal science;

- Provide assistance for institutional animal resource improvement projects to upgrade animal facilities and develop centralized programs for high-quality animal care that will comply with established standards;
- Support animal genetic repositories and breeding colonies to ensure the availability of valuable germplasm and animals needed for research on human diseases;
- Provide information related to animal research, sources of laboratory animals, and biological specimens; and
- Sponsor workshops and conferences on laboratory animals used in biomedical research.

The LASP budget totaled approximately $24.8 million in FY 1991. A summary of each element of the program is provided below.

Institutional Animal Facility Improvements. Healthy research animals are essential for obtaining meaningful and reproducible experimental data. The housing and care of these animals must be optimal to avoid the introduction of unwanted variables into the research. To assist institutions in their efforts to meet the animal care guidelines established by the Public Health Service and the requirements of the Animal Welfare Act, the LASP has continued to support projects to improve institutional animal facilities. Of the 113 animal resource improvement applications received for FY 1991, 46 projects were funded at a total of approximately $11 million. This support was used to renovate laboratory animal facilities, enhance the environmental conditions of the animals, upgrade animal caging, and improve sanitation equipment such as cage washers and autoclaves.

Training and Career Development. Postdoctoral specialty training in laboratory animal medicine and science continues to be a high priority for LASP. In FY 1991 the LASP supported the training of 77 veterinarians and 1 veterinary student in 17 institutional programs and 5 veterinarians on individual fellowships. These programs are designed to prepare individuals for careers in laboratory animal medicine, comparative pathology, and biomedical research.

The LASP also continued the Special Emphasis Research Career Award (SERCA) program. SERCA awards are made to veterinarians to enhance their development in biomedical research careers involving the use of laboratory animals. The award provides support for 5 years and is designed to provide the advanced training necessary for the awardee to become an independent investigator.

Diagnostic and Investigative Laboratories. The major objective of the Diagnostic and Investigative Laboratories is to improve laboratory animal health and enhance biomedical research at large medical research institutions. This is accomplished through the control of naturally occurring disease, studies of the etiology and pathogenesis of laboratory animal diseases, detection and characterization of new diseases, and development of new and improved laboratory animal models of human disease. Numerous important laboratory animal diseases and animal models have been identified, characterized, and described at these laboratories.

Animal Models and Special Colonies. Animals naturally develop a number of diseases that are similar to those that develop in people. Bacterial and viral infectious diseases, endocrine abnormalities, inherited metabolic imbalances, and tumors are among the maladies animals and humans share. Thus animals often provide good models for the study of diseases that afflict humans.

In 1991 CMP supported five animal model and special colony projects. The projects were devoted to identifying new models of human disease, improving the characterization of some already identified models, and increasing the availability of animals that have proved useful to biomedical researchers but cannot be obtained through commercial sources.

Animal models known to be helpful in the study of the etiology, pathogenesis, and treatment of human infectious, genetic, and malignant diseases include a wide variety of species. Investigators in LASP-sponsored programs have studied diseases of rodents, rabbits, cats, dogs, nonhuman primates, and marine animals to identify new models. Recently laboratories supported by CMP have employed molecular and biotechnical techniques to develop specifically targeted transgenic animal models for studying a variety of human diseases. One laboratory has transferred human genes into animal models to replace missing genes. Two laboratories have developed molecular genetic markers in nonhuman primates to perform preliminary DNA fingerprinting for identification and parentage determination.

Primate Resources. The LASP supported several studies dealing with the breeding, genetic characterization, diseases, and laboratory environment of nonhuman primates. The Caribbean Primate Research Center (CPRC) includes a large colony of rhesus monkeys

Antibody against the protozoan parasite Cryptosporidium *may protect both animals and humans against cryptosporidiosis. The infection causes severe diarrhea, weight loss, and abdominal cramps. Drs. Ronald Fayer (left) and Albert Guidry at the Agricultural Research Service in Beltsville, Maryland, who collaborate with CMP-funded scientists, collect colostrum that contains protective anti-*Cryptosporidium *antibody from the udder of a cow injected earlier with inactivated* Cryptosporidium.

Courtesy of Dr. R. Fayer

and several smaller colonies of other nonhuman primates. Maternal genealogical and demographic data have been collected continuously on the free-ranging rhesus colony on the offshore island of Cayo Santiago since 1956. This has resulted in the most complete life history information on any nonhuman primate population in the world. The CPRC also maintains 1,600 complete or nearly complete skeletons of 11 species of Old World and New World primates. Approximately 900 of the skeletons are of known identity, age, sex, matriline, social group, diet, and reproductive history. These skeletons are valuable for studying normal and abnormal skeletal development, the effects of different diets, and the differences between sexes. The colonies support a wide variety of research studies, including studies on obesity, diabetes mellitus, hypertension, pathology of joints and bones, and ocular diseases.

The CMP supports the largest breeding colony of squirrel monkeys in the United States, at the University of South Alabama in Mobile. Behavioral, biochemical, and cytogenetic studies to enhance the reproductive efficiency of the colony and to develop the squirrel monkey as an animal model are being conducted.

The genetic management of nonhuman primates to accurately identify parents and maintain heterogeneity in self-sustaining colonies is important for biomedical research. Four studies aimed at various aspects of genetic characterization and colony management of nonhuman primates were supported. In FY 1991 several projects were funded to study methods and environmental factors to enhance the psychological well-being of the nonhuman primates used in research. These studies should benefit the animals, help institutions comply with recent animal welfare legislation, and allay public concerns about using nonhuman primates for research.

Reference Centers. To provide reference material for biomedical projects the LASP supports two reference centers that collect tissues and other biological specimens from laboratory, domestic, and wild animals. The Registry of Comparative Pathology at the Armed Forces Institute of Pathology in Washington, D.C., maintains a collection of tissues and illustrative material that is available for research and research training. The registry conducts workshops, symposia, and short courses and prepares study sets and syllabi for loan. One important service provided to investigators is the consultation on comparative pathology.

The Facility for Cryopreservation of Murine Germplasm at the Jackson Laboratory in Bar Harbor, Maine, has pioneered techniques for freezing mouse embryos to preserve valuable strains of mice that might be needed for later research. This technique reduces the number of animals that must be maintained in the live state and helps to maintain the genetic integrity of inbred animals. Selected strains of valuable transgenic mouse embryos are also being cryopreserved for future use.

Other reference centers supported were primarily information centers. The Institute of Laboratory Animal Resources of the National Research Council, with the support of LASP and other government agencies, provides scientific and technical information about laboratory animal resources. It develops guidelines on laboratory animal care and use and conducts workshops on laboratory animal problems. Several thousand copies of the resulting proceedings are distributed to the scientific community each year. The *Laboratory Primate Newsletter* at Brown University and *New Listings* from the Primate Supply Information Clearinghouse at the Washington Regional Primate Research Center provide important information regarding the care, breeding, and supply of nonhuman primates for laboratory studies.

Research Highlights

Regional Primate Research Centers

Induction of AIDS in Rhesus Monkeys. The New England and Tulane Regional Primate Research Centers reported the first effective vaccination against simian immunodeficiency virus (SIV) in rhesus monkeys. More recently, investigators at the New England Regional Primate Research Center demonstrated that a molecularly cloned SIV can induce disease symptoms in rhesus monkeys that are identical to those of AIDS. Disease induction had not been reported previously for any molecularly cloned lentivirus. The complete genetic sequence of this pathogenic molecular SIV clone has also been determined.

The rhesus monkey model is currently being used to study the role of so-called nonessential genes for the virus life cycle in vivo and the significance of other genes for viral infectivity and persistence.

Emotional Stress and Hypertension. By conservative estimates 15 percent of Americans have hypertension. Most of them will die of diseases such as heart attack or stroke, which are directly related to hypertension. For 90 percent of these hypertensive individuals the cause is unknown. A large body of clinical literature suggests, however, that emotional stress, which involves a number of major neural systems within the brain, may be involved. Studies conducted at the Washington Regional Primate Research Center during the past 20 years have identified a small group of cells deep in the forebrain that appears to mediate cardiovascular effects of highly emotional behaviors.

Studies were also conducted in baboons to identify a characteristic pattern of cardiovascular responses to emotional stress. The responses include increased blood pressure and heart rate and decreased blood flow to the kidneys. The scientists then stimulated the brains of anesthetized animals with a fine needle-like electrode to see which sites would elicit the same pattern of cardiovascular responses. Among the identified sites was a diffuse collection of cells in the hypothalamus, a small structure located in the lower part of the brain.

Selective destruction of the nerve cells in the hypothalamic area controlling emotional response (HACER) abolished the cardiovascular response pattern characteristic of emotion. These findings suggested—and more recent studies have confirmed—that the cardiovascular effects of activation of forebrain areas involved in emotion are funneled through the HACER to the brainstem and spinal cord nerve centers that exert direct control over the end-organs of cardiovascular function.

Prevention of Thrombosis. Thrombosis—the formation of blood clots inside blood vessels—is a major life-threatening occurrence in humans, often as a complication of cardiovascular surgery. In a search for improved prevention and therapy for thrombosis, investigators at the Yerkes Regional Primate Research Center have evaluated numerous peptides and procedures in a nonhuman primate model. A chemically modified dodecapeptide consisting of 12 amino acids from the leech protein hirudin appeared to specifically prevent formation of the fibrin-rich type of thrombus found in veins. This provides a therapeutically attractive regimen for preserving normal platelet function when conventional anticoagulant therapy is contraindicated.

Laboratory Animal Sciences Program

Conference on Development of Transgenic Animal Model Resources. The purpose of the conference, which was held at the NIH in Bethesda, Maryland, was to explore the role of NCRR in supporting the development of transgenic animal model resources. The presentations discussed methods for preserving and storing embryos; nomenclature and database management of transgenic embryos; genetic mapping, biology, and gene targeting of embryonic stem (ES) cells; comparative assessment of species for transgenic animal model development; and methods for gene transfer. At the conclusion of the conference, the participants recommended that the NCRR support development and production of transgenic rats, rabbits, and chickens; development of an official nomenclature and database for transgenic animals; cryopreservation of transgenic embryos, gametes (partic-

Drs. Pelayo Correa (left) and Nicholas J. Persich, of Louisiana State University Medical Center in New Orleans, perform a gastroscopic examination to search for evidence of infection by Helicobacter *bacteria.* Helicobacter *causes gastritis in humans and is a potential cause of stomach ulcers and gastric cancer. CMP-funded veterinarians at the Massachusetts Institute of Technology in Cambridge found that the ferret may serve as an animal model for human gastric* Helicobacter *infection.*

Louisiana State University Medical Center

ularly sperm), and ES cell lines; improvement and expansion of existing transgenic production facilities; development of methods to detect gene integration in embryos after transfer; and techniques to increase ova production and recovery in larger species.

Cryopreserved Mouse Embryo Repository. The primary importance of this repository at the Jackson Laboratory in Bar Harbor, Maine, is the assurance it provides that valuable strains of mice will always be available to biomedical investigators. Inbred, mutant, and transgenic strains of mice used in research are frozen as eight-cell embryos. Many of these strains represent important animal models of human diseases. These mice are also used to study the mammalian genome. In addition to preserving irreplaceable murine germplasm, the repository helps to reduce the number of different strains that must be maintained by conventional breeding and thereby minimizes the likelihood of genetic drift. Currently the repository consists of 762,723 embryos from 859 strains of mice, 272 of which are available only in the repository. As the number of embryos in the repository continues to increase, so does the value of this important resource.

National Referral Center for Animal Models for Human Genetic Diseases. The center, which is located at the University of Pennsylvania in Philadelphia, serves as a national referral center to identify, characterize, evaluate, and disseminate animal models for human genetic diseases to qualified investigators. In addition, sperm and somatic cells from valuable models are cryopreserved and the DNA separated and stored for future molecular genetic studies. Last year 755 animals with clinical signs suggestive of an inherited metabolic disease were studied. These included 615 dogs, 84 cats, and small numbers of maned wolves, llamas, calves, and swine.

The referral center identified a family of giant schnauzer dogs with clinical signs of vitamin B_{12} deficiency and found an inherited deficiency of the muscle type phosphofructokinase in the English springer spaniel dog. In humans the latter condition produces a muscle disease called glycogenosis type VI.

A skin condition in a young male English setter dog was diagnosed as very closely resembling Hailey-Hailey disease in humans. This new model of inherited benign pemphigus has the same pathologic abnormalities and mode of inheritance as the human skin disease.

Biomedical Research Support Program

Program Description

The Biomedical Research Support Program (BRSP) encompasses three grant programs that help PHS-funded biomedical institutions meet critical research needs. These programs, which promote innovation, bolster research infrastructure, and strengthen science education at the precollege level, are the Biomedical Research Support Grant (BRSG) Program, the Shared Instrumentation Grant (SIG) Program, and the Minority High School Student Research Apprentice (MHSSRA) Program. The MHSSRA Program was expanded in FY 1991 to include high school science teachers. Institutions receiving these grants include graduate schools, health professional schools (medicine, dentistry, pharmacy, veterinary medicine, and nursing), not-for-profit research organizations, and hospitals.

In FY 1991 ADAMHA and NIH announced a joint initiative, the Science Education Partnership Award (SEPA), which aims to increase the science literacy both of students in grades kindergarten through 12 and of the general public. The NIH component of the program was administered by BRSP. Of the 200 applications received, 12 NIH awards were made for the development of model programs that span the gamut of science education, from a national video education program; a traveling AIDS museum exhibit; simple, hands-on laboratory experiments for elementary and middle school students; biotechnology research experiences for students and teachers; to health-promoting outreach programs for inner city and rural communities. If successful, these innovative pilot programs will be replicated across the country to increase the number of citizens knowledgeable about science.

Congressional appropriations for BRSP to fund the three grant programs and the administration of SEPA during FY 1991 totaled approximately $64.5 million.

The Biomedical Research Support Grant Program

The BRSG Program complements and enhances other NIH/PHS grant support for biomedical and behavioral investigations. It provides funds for small-scale, short-term support of research projects, using mechanisms that allow rapid response to unanticipated needs and opportunities. It responds to the need for preliminary explorations of new ideas, techniques, and approaches before competitive applications for regular research grants can be written. It answers the need for flexible funding that can allow an investigator to take advantage of new opportunities that could not be anticipated in previous budgets. To a limited extent it also responds to needs for emergency funding during temporary breaks in regular research funding. By meeting these needs BRSG funds enable research project grants to be used with greater precision and effectiveness.

To be eligible for the formula-based support from the BRSG Program an institution must have received at least three Public Health Service research grants totaling $200,000 or more during the previous Federal fiscal year. The size of a BRS grant is calculated by applying a formula to the level of the institution's PHS-supported research during the previous year. A total of 628 institutions received $22.3 million in BRS grants during FY 1991; the largest award was $131,201 to Johns Hopkins University School of Medicine in Baltimore, Maryland. Forty-nine grantee institutions received minimum awards of $5,000.

Types of critical needs unmet by project-oriented research grants but funded by BRSG include:

- **Pilot Studies.** Close to 6,600 innovative research ideas in early stages of development were funded by the BRSG Program in FY 1991. Research subjects included health studies of minorities, women, and underserved populations; biotechnology; neuroscience; disease prevention; and gene therapy.
- **Young Investigators.** BRSG funding helps new faculty members set up their laboratories; with BRSG seed money they initiate studies that may provide the basis for competitive project grant applications. More than 220 investigators who received BRSG funding in FY 1988 and 1989 were subsequently awarded 5-year FIRST (First Independent Research Support and Transition) awards totaling $19.6 million. FIRST awardees in those 2 years have since won 18 percent of the new regular research grants awarded by NIH.

- **Interim Support.** Sharp reductions in the number of new and competing NIH research grants have had a detrimental effect on many competitive renewal applications despite excellent priority scores or percentile rankings. Increasing numbers of these meritorious projects have been supported with BRSG funds on an interim basis, keeping productive programs alive during temporary breaks in funding and preventing expensive shutdown and startup procedures.

The BRSG Program provides no indirect costs, and grantee institutional panels are delegated the responsibility of making decisions in these low-cost research areas, saving time and money and minimizing the involvement of NIH staff.

The Shared Instrumentation Grant Program

Expensive, high-technology instrumentation is a prerequisite for cutting-edge health research. By providing sophisticated, complex instruments the SIG Program allows groups of NIH grantees to enhance the goals of their ongoing research programs. Rapid technological advances have opened new scientific areas and increased the rate at which research data can be obtained; the sensitivity of new analytical techniques and tools makes data more reliable. Examples of these new instruments include, but are not limited to, powerful new NMR imagers, coupled hybrid mass spectrometers, scanning laser confocal microscopes, and the latest in gene sequencing equipment.

To achieve significant research advances in a highly competitive, technologically oriented research environment, researchers require access to the unique capabilities provided by currently available elaborate instrumentation. Unfortunately, these new state-of-the-art instruments are very expensive (over $100,000) and cannot be provided through the regular research grant mechanism. The SIG Program offers a cost-effective solution to this problem since groups of NIH grantees (an average of eight users) can compete for an instrument in the $100,00 to $400,000 cost range on the basis of their need and the instrument's ability to enhance and extend the research goals of their ongoing NIH-funded projects. In FY 1991 the SIG Program received 401 grant applications requesting a total of $117.7 million. Of these, 139 applications were funded for a total of $32 million. The average SIG award was approximately $230,000, about the size of an average research project grant. Over 1,000 NIH-funded grantees were major users of the awarded instruments. Had these 1,000 users applied individually for the instrumentation through their research grants it would have cost eight times more and would not have led to the sharing or interdisciplinary interactions that occur at the institutional level. Nor would there be any cost savings if the individual instruments had to be maintained separately.

Several research highlights summarized below are NIH-funded research projects that required the unique capabilities of the instruments supported by the SIG Program.

Minority High School Student Research Apprentice Program

A key objective of the MHSSRA Program is to interest minority students in pursuing careers in health-related research and in health care. Demographic patterns indicate that such minority representation is becoming more and more urgent in the health-care professions, which include health science researchers and practicing physicians.

The MHSSRA Program motivates and stimulates interest in biomedicine by providing high school students with up to 8 weeks of hands-on experience in basic or clinical laboratories. The students learn the underlying concepts of molecular and cell biology, evolution, development and reproduction, heredity, and ecology by carrying out actual laboratory experiments as part of a scientific investigation. In this way the students' curiosity and understanding of scientific concepts reach far beyond that achievable through the ordinary classroom and textbook experience.

In FY 1991 the MHSSRA Program was extended to include a high school science teacher initiative. To qualify, a science teacher must be a member of a minority group or teach a significant number of minority students. The program is designed to provide high school science teachers with in-depth laboratory experience in biomedical sciences such as physiology, genetics, developmental biology, microbiology, and immunology during a summer internship at a local university, health professional school, or research organization. A summer internship offers these science teachers the opportunity to update their skills, learn modern research techniques, and broaden their knowledge of fundamental principles of biomedical sciences—an opportunity that can translate into a lively enthusiasm for science education in the classroom. The long-range goal is to establish year-round linkages between biomedical scientists and science teachers that will foster continued mentoring and encourage young students to seek careers in bioscience.

Allocations to the MHSSRA Program more than doubled in FY 1991. From $3.2 million

Leticia Washington (right) of the Minority High School Student Research Apprentice Program (MHSSRAP) conducts an experiment at Georgetown University Medical Center in Washington, D.C., with her mentor, associate professor of pharmacology Dr. Barbara Bayer.
John Spaulding, Georgetown University Medical Center

in FY 1990 the program reached $8.3 million in FY 1991; 391 biomedical research and training institutions participated. The funding supported 468 positions in the MHSSRAP science teacher initiative. Each MHSSRAP teacher position was funded at $5,000, which included supplies or other needs but no indirect costs.

MHSSRAP student positions numbered 2,970 in 1991, a dramatic increase over the 1,116 apprenticeships filled in FY 1989.

Research Highlights

Brain Cells in Culture Regularly Emit Fertility-Control Hormone. Nerve cells from mouse brain growing in tissue culture secrete gonadotropin-releasing hormone (GnRH) in pulses every 20 minutes, just as they do in the intact mouse brain. This discovery by BRSG-funded California scientists is the first evidence that mouse brain cells can release GnRH, a fertility-controlling hormone, independently—without input from any other type of cell. This could lead to novel treatments for infertility and new methods of controlling conception.

GnRH is also regularly secreted by human nerve cells in the same part of the brain, the hypothalamus, but more slowly than by the mouse brain cells. Hypothalamic cells in sexually mature women normally release GnRH every 1 to 4 hours, changing with the menstrual cycle. GnRH is released in males as well as females; the start of the pulsed GnRH release stimulates the onset of puberty in both sexes. As adolescents mature sexually, GnRH triggers a hormone cascade that leads to production of sperm or eggs.

Research on the GnRH secretion by cultured brain cells began as a BRSG-funded pilot project at the Salk Institute in La Jolla, California, in 1988. Investigations are continuing there and at the University of California in San Francisco.

Natural Tranquilizer in Neurologically Impaired Liver Disease Patients. At least two natural benzodiazepines (BZ) and other compounds with BZ-like tranquilizing activity have been found in blood, urine, and cerebrospinal fluid of neurologically impaired liver disease patients, according to BRSG-funded investigators at Case Western Reserve University in Cleveland, Ohio.

Synthetic BZ (commonly known by the trade name Valium) exerts its effect by augmenting the neurotransmitter GABA in blunting the actions of certain nerve cells and initially decreasing anxiety, relaxing muscles, and controlling epileptic seizures.

Cirrhotic patients who have the type of brain dysfunction called hepatic encephalopathy, which is now known to be associated with the presence of natural BZ compounds, doze frequently during the day and some become disoriented and comatose. The investigators note that this encephalopathy is totally reversible by flushing out the gastrointestinal (GI) tract and increasing the acidity of GI contents. With the source of hepatic encephalopathy now identified, administration of an anti-BZ pharmaceutical may be a possible alternate approach. One such compound has already been tested clinically by several groups but with inconclusive results.

Bone Growth in Rats Stimulated by Negatively Charged Beads. Negatively charged microscopic beads of Sephadex, a starch-like material, stimulated bone growth in rats when implanted in bony defects of the jaw and cranium and when applied to the rats' nasal bone surfaces. A BRSG award to the Washington University School of Dental Medicine in St. Louis, Missouri, funded a pilot study that led to the recently published findings.

The investigators found that new bone forms only if negatively charged beads establish contact with existing bone or are placed inside a bony environment. The scientists believe that growth factors may be attracted by the charged particles. They suggest that the negatively charged beads might one day be useful in transporting growth factors to specific sites and keeping them in place to facilitate bone growth.

The investigators note that clinical devices generating electrical fields have been available for about 10 years to treat bony nonunions; however, these devices are too bulky to be practical in the craniofacial skeleton. Although it is not yet clear how effective negatively charged beads or other negatively charged implants might be in clinical applications, the scientists suggest that further investigations are warranted, especially when directed at possible periodontal and orthopedic applications for the nation's aging population.

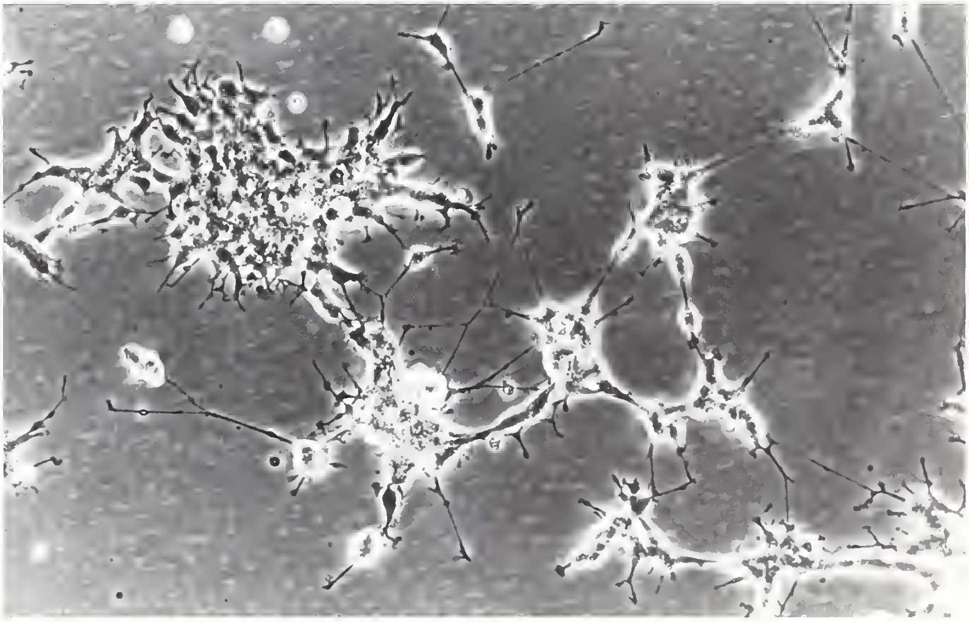
Virus Transmitted by Mosquitoes Causes Birth Defects in Sheep. BRSG funding to investigators at Texas A & M University at College Station led to the recent discovery that severe congenital malformations in sheep can be caused by Cache Valley virus (CVV). A member of the Bunyaviridae virus family, CVV was already known to be transmitted by insects, primarily mosquitoes. It was also known to infect various Western hemisphere mammals, including humans and sheep.

The Texas veterinarians and parasitologists investigated a large outbreak of congenital abnormalities in a flock of sheep in San Angelo, Texas; 26 percent of the lambs had been born with abnormal limbs or other musculoskeletal malformations or brain abnormalities. The ewes that gave birth to malformed offspring all had antibody against CVV. Forty-five of the 75 ewes with normal lambs also tested CVV positive. Only 5 percent of the flock had been CVV antibody-positive the previous year, showing how easily CVV infection spread during the heavy rains and mosquito infestation of the following lambing season.

The Texas investigators' discovery is the first indication that CVV can cause congenital abnormalities in sheep. It is not known whether CVV causes birth defects in humans or other animals; the investigators suggest that a large epidemiologic study may be appropriate.

Infants' Difficulties in Dissolving Blood Clots Explained. At least 1 percent of distressed newborns in certain intensive care units develop blood clots in conjunction with catheters that have been inserted to deliver medications or to remove blood samples. The vast majority of these babies fail to respond to thrombolytic therapy, BRSG-funded pediatricians recently found, because the blood of newborns has markedly less plasminogen—a protein required to dissolve blood clots—than do older children and adults.

The BRSG-funded investigators, working at the University of Arizona Health Sciences Center in Tucson, found that naturally occurring plasminogen activity is 63 percent lower in newborns than in adults. In addition, in vitro studies of blood specimens showed that newborns required five times the usual adult dosage of the drug called tissue plasminogen activator (tPA) to generate the same concentration of the clot-dissolving protein plasmin as that normally found in adult blood plasma.



Cultured mouse brain cells regularly release an important fertility-controlling hormone, gonadotropin-releasing hormone (GnRH), as do human cells from the brain area called the hypothalamus. The cells' synchronous release of GnRH was discovered by BRSG-funded scientists at the Salk Institute in La Jolla, California, and the University of California, San Francisco.

Dr. Pamela Mellon, Salk Institute

Natural tranquilizers that are chemically similar to the benzodiazepine drug Valium have been detected in the blood, urine, and cerebrospinal fluid of neurologically impaired liver disease patients. Dr. Kevin D. Mullen and research assistant Kristine Kaminsky-Russ at Case Western Reserve University in Cleveland, Ohio, are analyzing patients' body fluids to determine the source of the compounds.

Case Western Reserve/MetroHealth Medical Center

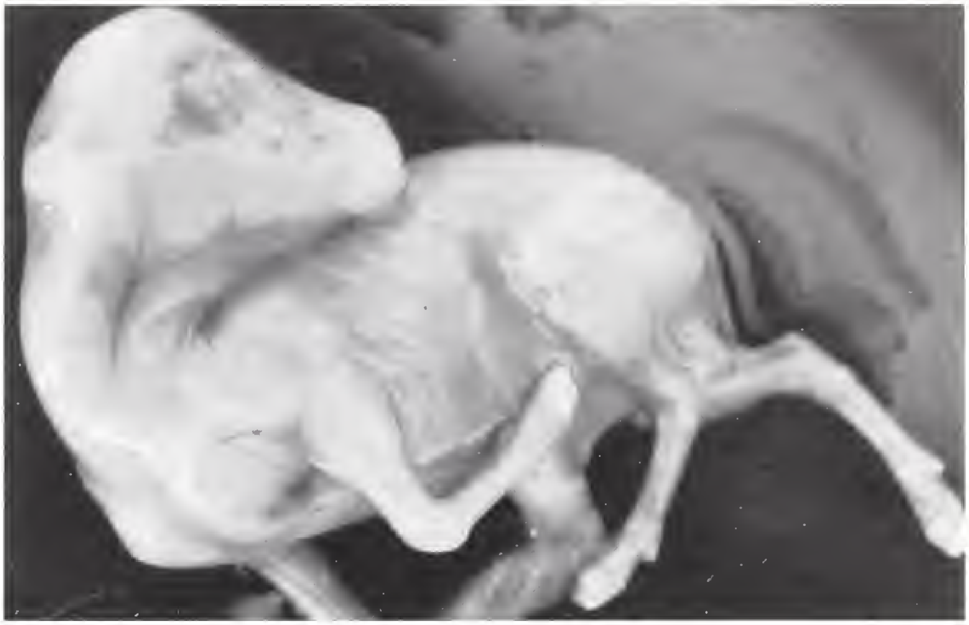
The scientists suggest that plasminogen be considered a developmental protein in newborns; the level of plasminogen increases gradually until it reaches adult levels at 6 months. The investigators suggest that distressed newborns who develop blood clots might respond to treatment with higher dosages of plasminogen-activating drugs. They plan to further explore the neonatal blood clot-dissolving mechanism in collaboration with an international team of neonatologists.

Kidney Function Probed With Interactive Laser Cytometry. A positive biochemical urine test for albumin provides a sensitive marker of the onset of kidney disease. A better understanding of the mechanism of albuminuria, or albumin loss into the urine, may be a key to preventing and treating kidney disease. For many years albuminuria has been attributed to altered permeability of the basement membrane that lines the glomeruli of the kidney. New light is being focused on the problem with the aid of the interactive laser cytometer, awarded through a $249,000 SIG to 13 NIH-funded researchers in various departments at the University of Minnesota School of Medicine in Minneapolis. In contrast to flow cytometers, this relatively new instrument analyzes, selects, and sorts anchored cells, thus permitting single and multiple serial measurements on selected individual cells. Two- and three-dimensional confocal imaging can also be carried out.

Now with a novel technique to isolate glomerular basement membranes—plus mathematical models and paradigms of glomerular filtration—and through the use of all the features of the interactive laser cell cytometer awarded by the SIG Program, the NIH-supported investigators have challenged the almost exclusive role of basement membrane permeability as a factor in the filtration of albumin and other proteins. In 1991 they reported to the American Society of Nephrology that glomerular cells themselves, not the basement membranes, are the major determinant of permeability.

Inner Workings of Lung Seen by Transmission Electron Microscopy. An SIG award of $184,000 to the University of North Carolina at Chapel Hill made it possible to purchase a new transmission electron microscope. More than 20 investigators who have research grants from several NIH institutes have used the new instrument to study the development of lung epithelial cells, which line the airways, in patients with cystic fibrosis and other respiratory diseases. The state-of-the-art electron microscope provides greater resolution and contrast than do older instruments and is equipped with a tilting goniometer that allows optimal viewing of selected cells. This modern electron microscope was also necessary for the NIH-funded researchers to gain insights into whether patterns of early airway epithelial development are recapitulated during regeneration following injury at the cellular and molecular levels. The electron microscope has made possible a better understanding of the reorganization of this inner respiratory epithelium during acute injury, such as damage during viral upper respiratory tract infections, and after acute exposure to certain air pollutants.

Novel T-Cell Found in Mice With Graft-Versus-Host Disease. Thanks to special imaging and cell sorting instrumentation, several NIH grantees at Harvard Medical School in Boston, Massachusetts, have identified a novel T-cell in the target organs of mice that developed graft-versus-host (GVH) disease after transplantation of foreign bone marrow. GVH dis-



BRSG-funded scientists at Texas A & M University at College Station have traced an outbreak of birth defects in sheep to infection by the Cache Valley virus (CVV). CVV, a member of the Bunyaviridae family, is transmitted primarily by mosquitoes and infects humans and other mammals in the Western hemisphere. The photo shows a fetus with arthrogryposis—a malformation in which the joints are fixed in abnormal positions; this is particularly evident in the left foreleg.

Courtesy of Dr. E. W. Collison, Texas A & M University

ease is a serious and potentially fatal complication of organ transplantation between incompletely matched individuals. The Harvard scientists could not have made their discovery without using the interactive laser cytometer and other accessories purchased through an SIG award of $245,000.

The investigators point out that cells having the same characteristics as the newly identified T-cell are the predominant lymphocytes in the epidermis, or outer layer, of skin. These cells have also been detected in the spleens of mice with GVH disease 3 weeks after transplantation but are not found in transplanted mice without GVH disease.

The scientists found that the cells could cause the epidermal damage characteristic of GVH disease when transferred into other mice; the cells are therefore considered effector cells of cutaneous GVH disease.

Circular Dichroism System Aids in Understanding the Folding of Proteins. Studies facilitated by an SIG award of $112,000 to Pennsylvania State University have led five NIH-supported investigators in the departments of molecular and cell biology and chemistry to a better understanding of how a protein's folding influences its activity. The SIG award permitted purchase of a circular dichroism spectropolarimeter, a new instrument that allows detection of the secondary and tertiary structure of proteins. Access to this instrumentation has allowed NIH-funded researchers to develop new ideas concerning the folding process and the effects that changes in the amino acid sequence can have on the secondary protein structure.

The Pennsylvania scientists used a stopped-flow accessory on the equipment to probe very early events in the folding of the protein dihydrofolate reductase, an important enzyme in the body's metabolic processes. Their results indicate that an intermediate enzyme structure appears within 10 milliseconds after the beginning of the folding process. They suggest that this entity, whose counterparts have also been observed in a number of other proteins, plays a critical role in the folding mechanism. The investigators also state that no other current technique would have permitted this observation.

Other NIH-funded researchers at Pennsylvania State University have used the circular dichroism system to monitor the effects of simple and complex changes in the amino acid sequence of dihydrofolate reductase. They found that significant structural changes in the protein occur as a result of such protein engineering efforts.

How Environmental Toxins Interact With DNA. A $400,000 SIG award to Vanderbilt University School of Medicine in Nashville, Tennessee, permitted the Center for Molecular Toxicology to purchase a powerful 500 MHz NMR spectrometer system costing $698,000. NIH-funded researchers from the chemistry, biochemistry, and pharmacology departments have found this instrument to be essential for learning how toxic chemicals damage mammalian DNA and initiate cancers, birth defects, and other abnormalities at the molecular level. The 500 MHz NMR interfaced to a computer graphics workstation has permitted newer and faster one-dimensional and two-dimensional data acquisition and processing capabilities not previously available, allowing molecular models to be graphically visualized in various planes and under different conditions.

Much of the research to date has centered on studying modifications of DNA by benzo[a]pyrene, a cancer-causing component of tobacco smoke, and by the industrial chemical styrene. The latter chemical, ubiquitous in contemporary society, is believed to pose a threat to the chemical industry workers who produce it.

DNA interactions with aflatoxin, a toxic compound formed by mold that contaminates foodstuffs such as corn and peanuts, is also being examined with the aid of the NMR spectrometer.

Teacher at Indian Reservation School Joins New MHSSRAP Initiative. A science teacher on the faculty of Tohono O'odham High School in Sells, Arizona, assisted in clinical research at the University of Arizona (UA) Heart Center in Tucson for 8 weeks during the summer of 1991. With a UA immunologist as his mentor, the teacher coupled his computer skills with audiovisual techniques in research to develop visual methods of detecting early signs of rejection in heart transplant patients and in laboratory animals.

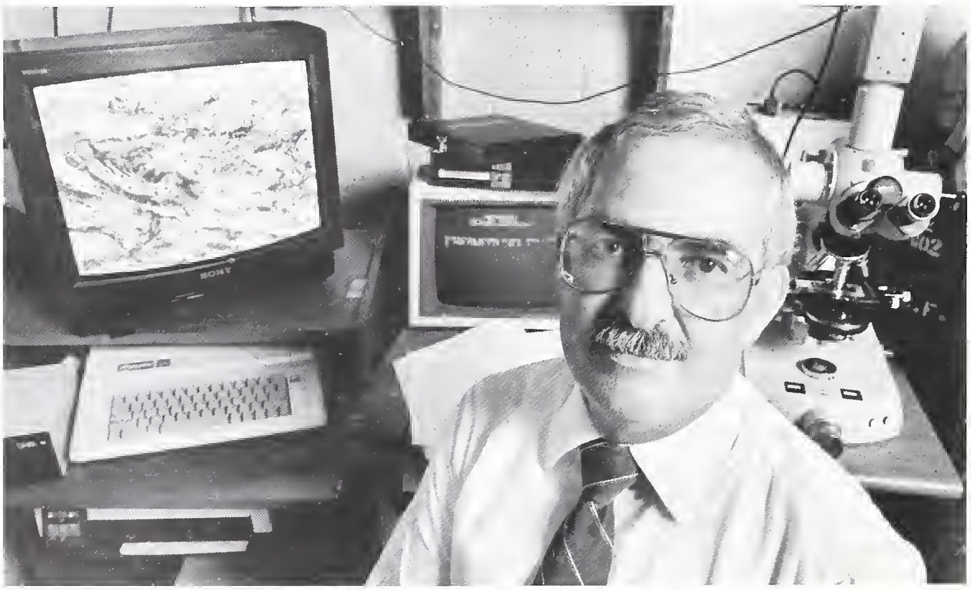
A Hispanic American, the teacher is one of three science teachers selected from 16 local teachers who applied for the summer position at UA made possible by the new MHSSRAP Teacher Initiative. He teaches general science and biology on the Tohono O'odham reservation, which is named for the Indian people once called the Papago. The teacher is enthusiastic about his MHSSRAP experience and expects his new teaching materials, and a planned followup by his immunologist mentor, to help convey to Tohono O'odham High School students the relevance, attainability, and excitement of a career in biomedicine.

The selected teacher was just 1 of 468 science teachers nationally who participated in the new MHSSRAP Teacher Initiative during 1991. The University Heart Center in Tucson is a major heart transplant center, having performed 278 such procedures since 1979.

MHSSRAP Student Wins in 1991 Westinghouse Science Talent Search. During 1991 an Ames, Iowa student in the MHSSRAP was 1 of 40 finalists in the country's most prestigious precollege science competition, the 50-year-old Westinghouse Science Talent Search (WSTS).

The 17-year-old Ames High School senior had been selected in 1990 to serve as an apprentice in the MHSSRAP at the plant pathology laboratory of Iowa State University. She devoted her MHSSRAP apprenticeship primarily to a project on the genetic engineering of pest-resistant poplar trees; in this context she helped develop a time-saving test to assess whether genes conferring pest resistance were transferred from potatoes to poplars. She attributed her 1991 selection by the WSTS, which was accompanied by a $1,000 award, partly to her research paper on the electrical propagation of proteinase inhibitor II in poplar hybrids.

The student attended the American Phytopathological Society meetings in August 1991 and then entered Iowa State University College of Agriculture with academic recognition and a Scholarship for Excellence covering tuition for 4 years. The former MHSSRAP apprentice plans to major in genetics; her career plans include both scientific research and science teaching.



One of NCRR's new MHSSRAP Teacher Initiative awards was presented to Joseph Bustamante, who participated in research at the University of Arizona Heart Center in Tucson during the summer of 1991. Mr. Bustamante used audiovisual techniques to develop methods to detect early signs of rejection of heart transplants. Mr. Bustamante, who teaches science at an Indian reservation high school in Sells, Arizona, plans to use computer skills and his hands-on experience in cardiology and immunology laboratories to demonstrate the excitement of a career in science to his classes.

John Charley, University of Arizona

Biological Models and Materials Research Program

Program Description

The Biological Models and Materials Research Program (BMMRP) supports and promotes increased development and use of nonmammalian model systems in biomedical research. Such model systems may help conserve the limited supply of mammalian animal resources and will allow studies that are not feasible in higher organisms.

A major effort of the BMMRP is to expand the variety and utility of models that provide information useful for understanding human physiology and diseases. The model systems include invertebrates, lower vertebrates, cultured cells, and nonbiological systems such as computer simulations and mathematical models. In FY 1991 the BMMRP funded 31 research projects and resource facilities with its total $8.1 million appropriation, a 53 percent increase over the $5.3 million appropriation used to carry out BMMRP's objectives in FY 1990.

To advance its objectives during 1991 BMMRP solicited proposals from the research community to develop high-connectivity nonmammalian models. These are models utilizing organisms or biological systems about which a large body of knowledge is available. The organisms are connected or crosslinked by their sharing of characteristics and properties with other systems across many taxa. The BMMRP has also initiated a small grants program for pilot projects and feasibility studies for developing nonmammalian models.

The BMMRP currently funds 20 research projects using nonmammalian model systems. Among them are several grant-funded high-connectivity models, including the use of frogs to investigate immune mechanisms related to the major histocompatibility complex; studies of fruitflies to determine mechanisms by which growth factors contribute to embryonic development; research using turtles to study coordinated hormone regulation of apolipoprotein metabolism; and exploration of steroid hormone action in yeast cells.

The BMMRP supports several projects focused on developing transgenic fish as biomedical research models. A grant to the University of Minnesota supports studies of the thyroid hormone receptor gene(s) C-*erb-A* in transgenic zebrafish. Regulation of the expression of this gene is known to be critical to normal development of all vertebrates, including humans. Transgenic research was also conducted during 1991 at the Southwest Texas State University in San Marcos; these studies using the medaka, a small Japanese freshwater fish, are designed to understand events leading to tumor formation.

Nonbiological models such as computer, mathematical, and physical models supported by the BMMRP include models to clarify aspects of the immune system and mammalian kidney function; a mathematical model of lung vascular function; and a computer model to predict how sodium and potassium are transported into and out of the heart under normal physiologic conditions and in heart disease.

The BMMRP supports a number of resource centers, including facilities that supply materials critical to progress in biomedical research. These resources assist individual scientists by providing biomaterials and organisms; maintaining normal and abnormal cells of many species; establishing methods for laboratory culture of nonmammalian marine organisms; and developing collections of valuable biomaterials such as cloned genes, DNA probes, and human chromosome libraries. Such centers include the American Type Culture Collection (ATCC) in Rockville, Maryland; the National Disease Research Interchange (NDRI) in Philadelphia, Pennsylvania; the Caenorhabditis Genetics Center (CGC) in Columbia, Missouri; the Marine Biomedical Institute in Galveston, Texas; the Yeast Genetic Stock Center (YGSC) at the University of California, Berkeley; the Repository of Mice and Human DNA Probes and Human Libraries located at the ATCC; and the National Cell Culture Center in Minneapolis, Minnesota.

The BMMRP also supports projects to develop new biomaterials and provides core support for established centers that make nonmammalian model systems more accessible for research. For instance, the program supports an effort at the University of British Columbia in Vancouver, Canada, to provide the researchers who study the worm *Caenorhabditis elegans* with a comprehensive genetic resource, in particular a genetic toolkit of chromosomal balancers, rearrangements that suppress genetic recombination. Genetic stocks of C. *elegans*, which are supplied by CGC, are expected to help in isolating and mapping mutations in any part of the C. *elegans* genome.

Also funded is the Center for the Study of Human Polymorphism (Centre d'Etude du Polymorphisme Humain), or CEPH, in Paris, France, which collaborates with the ATCC in assembling human DNA probes for distribution to Human Genome Project researchers in their effort to localize all of the individual human genes.

During 1991 BMMRP awarded a grant to the California Institute of Technology in Pasadena to develop methods to produce highly inbred lines of a purple sea urchin called *Strongylocentrotus purpuratus*. This marine organism is an important model system for understanding molecular and cellular processes in embryonic development. Inbred strains of S. *purpuratus* with known genetic differences or polymorphisms will be made available for a variety of research investigations on fertilization and early embryonic life.

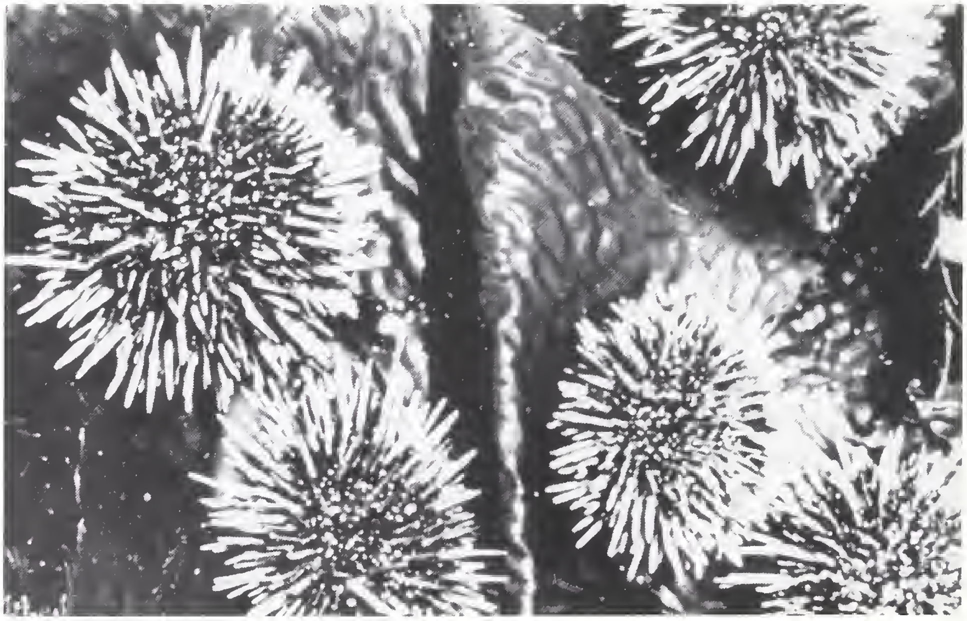
Program Highlights

Caenorhabditis Genetics Center. During 1991 the BMMRP continued its support of the Caenorhabditis Genetics Center (CGC) in Columbia, Missouri. The Center currently maintains more than 1,570 mutant strains of the nematode C. *elegans*, which is a model proving ground for gene sequencing by the Human Genome Project. The CGC distributed over 1,298 strains in FY 1991 to the C. *elegans* research community. At a BMMRP-cosponsored conference on C. *elegans* at the University of Wisconsin in Madison, over 500 participating scientists learned that sequencing the entire C. *elegans* genome, estimated at 100 million base pairs, is ahead of its projected completion by the year 2000. Strains of this tiny roundworm are already being used by hundreds of researchers, including geneticists, neurobiologists, developmental biologists, and environmental toxicologists.

One investigator reported at the conference that the millimeter-long roundworm may be effective in environmental monitoring for mutagenic chemical pollutants. Working at the Lawrence Berkeley Laboratory in Berkeley, California, the scientist found that mutagenic chemicals triggered genetic changes in paralyzed worms of a mutant strain that resulted in motile progeny. This work suggests that this multicellular organism, which ordinarily lives in soil, might be an equally effective biomonitor for soil, water, and air contamination. The scientist and her colleagues are currently testing this motile-worm assay on sediment samples from San Francisco Bay.

Other scientists reported in 1991 on C. *elegans* as a potential model system for the study of aging. They discovered that a mutation in one of the genes controlling aging in C. *elegans* resulted in a 65 percent increase in the roundworm's mean lifespan and a 110 percent increase in its maximum lifespan. This same mutation apparently also was responsible for a fivefold decrease in the number of progeny produced over the life of the organism. These and related studies are expected to give geneticists clues about the contribution of individual genes to the normal and pathologic aging of humans.

Neurotransmission: Lessons Learned From Marine Life. Researchers at the University of California in Santa Barbara are studying the red abalone sea snail named *Haliotis* and symbiot-



The purple sea urchin Strongylocentrotus purpuratus *is an important model for studying fundamental molecular and cellular processes in embryonic development. BMMRP-funded scientists at the California Institute of Technology in Pasadena are developing methods to produce highly inbred lines of this marine animal.*

Pat Leahy, California Institute of Technology

At the BMMRP-funded Center for Alternatives to Animal Testing at the Johns Hopkins University School of Hygiene and Public Health in Baltimore, Maryland, Drs. Joanne Zurlo and John M. Frazier evaluate results of an in vitro toxicity assay.

The Johns Hopkins University

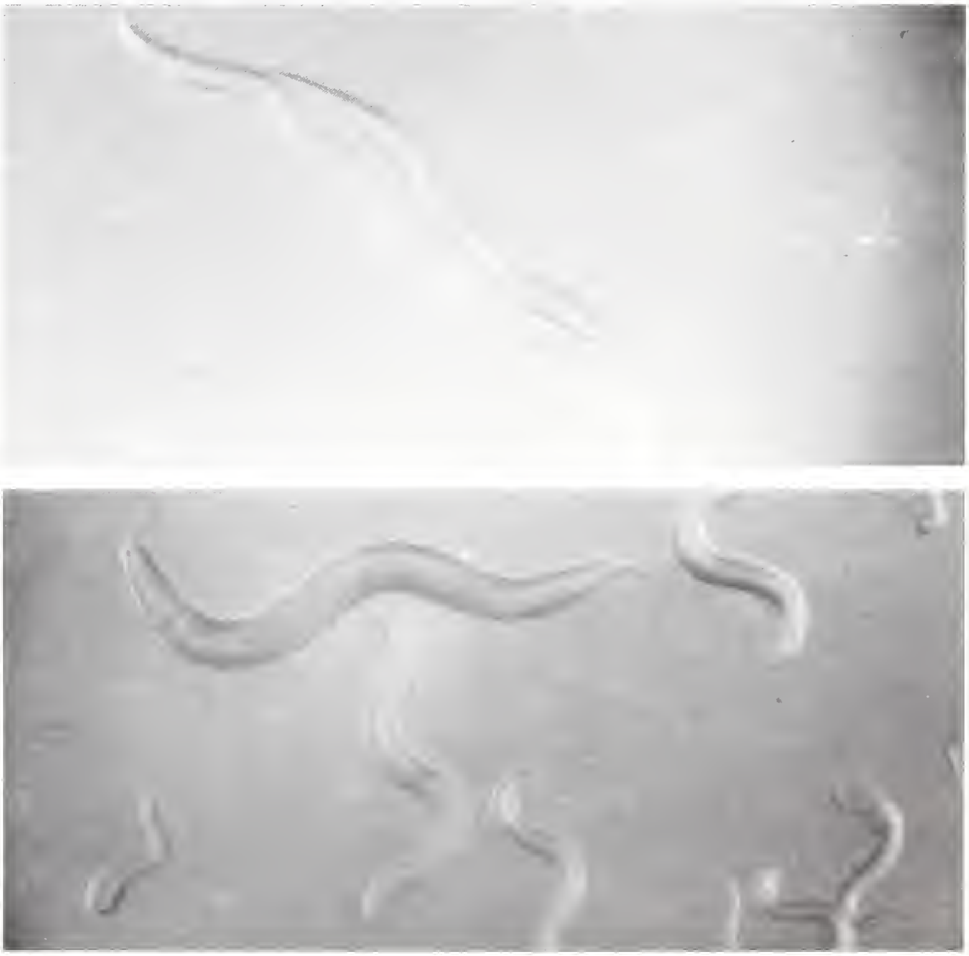
ic neighboring red algae to understand fundamental processes of chemosensory recognition and other phenomena associated with nerve signal transmission.

The scientists found that a small peptide isolated from coralline red algae has a structure similar to gamma-aminobutyric acid (GABA), a major inhibitory neurotransmitter in the human brain. Contact with the algae's GABA-like peptide gave the free-swimming abalone larvae a powerful neurochemical signal to settle down on algae-crusted rocks and begin their metamorphosis into adulthood. The investigators carried out extensive molecular studies of the peptide receptor and note that this is the first time a chemosensory receptor has been characterized at the biochemical level.

Hormones and Ovarian Cancer Cells. Human ovarian cancer cells supplied by the National Disease Research Interchange (NDRI) contributed to endocrine research conducted during 1991 at the University of Nebraska in Omaha. A Nebraska scientist investigating interrelationships between hormones and cancer studied surgically excised cell specimens and found that the cells continued to secrete the female hormones estradiol and progesterone in tissue culture. To evaluate the cultured cells' response to human gonadotropic hormones, the scientist added follicle-stimulating hormone (FSH) and another gonadotropin to the cell cultures. Approximately 30 percent of the cell cultures responded to the gonadotropic hormone stimulus with increased production of estradiol and progesterone. These investigations make it possible to study the hormone-secreting cells under well-controlled laboratory conditions.

DNA Probes To "Fingerprint" Human Cell Lines. The American Type Culture Collection in Rockville, Maryland, is a central repository for collections of normal and abnormal mammalian and bacterial cells, fungi, protozoa, and algae. For distribution to scientists worldwide, the center also maintains collections of viruses from animal, bacterial, and plant sources and libraries of plasmids and other DNA useful in genetic studies.

Researchers at the ATCC, in collaboration with scientists at the Frederick Cancer Research Center and the National Cancer Institute, recently developed a method to accurately and reliably identify and distinguish between individual human cell lines. The new method uses a set of DNA hypervariable probes that produce a "fingerprint" of each cell line. The procedure allows the rapid and accurate identification of most human cell lines.



Partially paralyzed roundworms of a mutant strain of C. elegans *(top) produce motile progeny when exposed to mutagenic chemicals (bottom). According to scientists at the Lawrence Berkeley Laboratory in Berkeley, California, the phenomenon may lead to a motile-worm assay for chemical contamination of water, soil, and air. The BMMRP-funded Caenorhabditis Genetics Center in Columbia, Missouri, serves as a clearinghouse for the acquisition, storage, and development of* C. elegans *mutants.*

Courtesy of Dr. Susan Anderson, Lawrence Berkeley Laboratory.
Photography: Bill Love

Research Centers in Minority Institutions Program

Program Description

Research excellence in a biomedically oriented center of learning is achieved with an appropriate mix of scientific creativity, institutional stability, and the necessary infrastructure. The latter requires an expert faculty with time for thoughtful research; modern, well-equipped laboratories and related facilities; and state-of-the-art instrumentation.

Most minority institutions functioned for many decades without the much needed research infrastructure. The Research Centers in Minority Institutions (RCMI) Program was mandated by Congress in 1985 to strengthen the scientific excellence and competitiveness of minority institutions so that they might effectively contribute to NIH research addressing the health needs of the entire population of the United States.

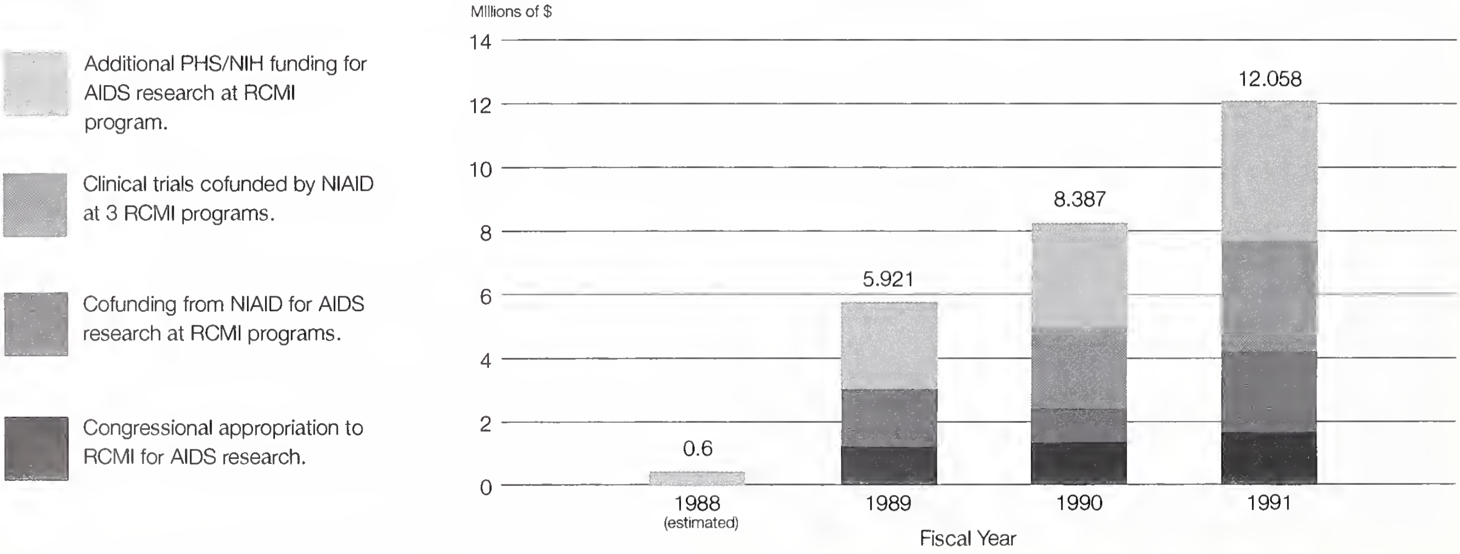
At present, the RCMI Program grantees are engaged in a critical effort to confront diseases—including cardiovascular diseases, diabetes, AIDS, and certain forms of cancer—that disproportionately affect Americans in minority communities. RCMI awards are designed to strengthen human resources and physical facilities and equipment. New faculty members are recruited, researchers receive advanced training in new techniques, laboratories are renovated, and biomedical research capacity is improved by the availability of new laboratory instrumentation, as well as specialized facilities.

RCMI funding is available to institutions whose minority enrollments exceed 50 percent. To be eligible for RCMI support an institution must award either an M.D., D.D.S., D.V.M., or an equivalent health professional degree; or it must have a doctoral degree program in at least one of the health-related sciences.

In 1991 there were 17 RCMI programs ongoing at 7 medical schools, 3 colleges of pharmacy, 6 graduate schools, and 1 school of veterinary medicine. The grantee institutions are located in Alabama, California, the District of Columbia, Florida, Georgia, Hawaii, Louisiana, New York, Puerto Rico, Tennessee, and Texas.

During FY 1991 funds totaling almost $25 million (including $2.7 million from NIAID and other NIH sources) were awarded to support the RCMI programs. Awards included 5-year renewal grants to Morehouse School of Medicine in Atlanta, Georgia; the University of Hawaii at Manoa in Honolulu; the University of Puerto Rico Medical Sciences Campus in San Juan; and the Universidad Central del Caribe in Bayamon, Puerto Rico. Tennessee State University in Nashville and Texas Southern University in Houston competed successfully for 3-year renewals of grants.

Funds Awarded to Support AIDS Research at Research Centers in Minority Institutions



Drs. Darlene Dixon and Curtis Parker (foreground) discuss their research presentations during a break at a symposium on environmental health sciences and toxicology at Clark Atlanta University in Atlanta, Georgia. The symposium was sponsored by the RCMI Program and the National Institute of Environmental Health Sciences.

Clark Atlanta University

During 1991 collaborations continued with the National Institute of Allergy and Infectious Diseases (NIAID), particularly in research on AIDS, and with the National Center for Human Genome Research (NCHGR) on genetic diseases and conditions that mainly affect minority Americans. At Howard University College of Medicine in Washington, D.C., RCMI-supported scientists are cooperating in NCHGR's program to create a resource of reference families from the African-American population. The long-range goal of this initiative is to define genetic differences that might distinguish DNA of African-Americans from that of Americans of different ethni-city and with different disease susceptibilities.

During FY 1991 eight institutions with RCMI programs participated in NIH-funded research on AIDS or AIDS-related diseases. The first RCMI International AIDS Symposium was held at Morehouse School of Medicine at the close of FY 1990; it generated several collaborative efforts of American scientists with investigators in African and Latin American countries that are disproportionately affected by the worldwide AIDS epidemic. The second annual International AIDS Symposium sponsored by the RCMI Program was also held in Atlanta and hosted by Morehouse. Reports were presented on AIDS epidemiology, clinical research, education and training efforts to counter AIDS, research to develop a vaccine, and approaches to prevention and treatment.

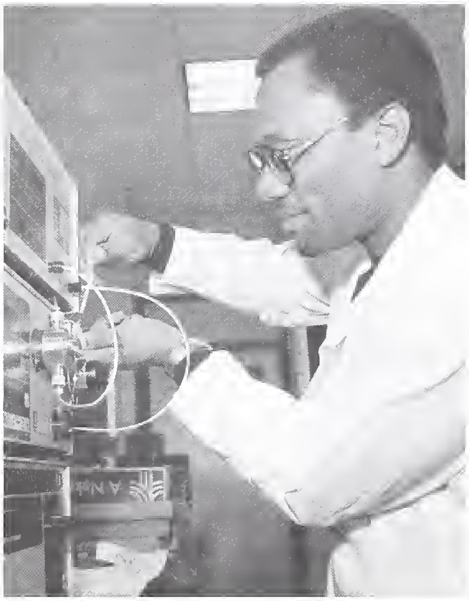
RCMI's program to conduct AIDS-related research at minority institutions continues to be greatly facilitated by transfer of funds from the NIAID and by other awards. Support for AIDS research at RCMI centers has grown from $600,000 in FY 1988 to approximately $12 million in FY 1991.

A significant new collaboration was also undertaken with the National Institute of Environmental Health Sciences (NIEHS) on environmental health and toxicology issues.

A 2-day RCMI-funded meeting on cardiovascular diseases in minority populations was held at Howard University, with NCRR and the National Heart, Lung, and Blood Institute (NHLBI) cosponsoring. Cardiovascular risk factors and disease prevention were a focus of the meeting, as were molecular biology studies designed to identify and characterize genetic factors that might serve as markers of high risk.

To reduce the risks of heart disease and diabetes among native Hawaiians an RCMI grant was awarded during FY 1991 to the University of Hawaii. Native Hawaiians, who have an increased prevalence of obesity, smoking, hypertension, glucose intolerance, and high cholesterol levels, will be studied in three settings: urban Honolulu; rural Molokai; and a transitional area on the island of Oahu. Assessment of health care promotion, disease prevention, and primary health care services will be undertaken in collaboration with a community-based organization that serves the indigenous Hawaiian population.

A symposium on environmental concerns held at Clark Atlanta University in Atlanta, Georgia, was cosponsored by RCMI and NIEHS. It focused in part on identifying and measuring human exposure to lead and inhaled toxic particles and gases; the use of molecular technology to test for viral and bacterial contaminants in water; and biochemical mechanisms involved in the depigmentation of human skin and hair by accidental or occupational exposure to benzene or similar chemicals.



Dr. Mofolorunso Enigboken of the RCMI at the College of Pharmacy and Health Sciences, Texas Southern University in Houston, conducts research on drug metabolism.

Texas Southern University

Involvement of Neuropeptide Y in Blood Pressure Regulation. A team of scientists at the Universidad Central del Caribe School of Medicine in Bayamon, Puerto Rico, have investigated how a neurotransmitter, known as neuropeptide Y (NPY), may influence the contractility and relaxation of blood vessels. The investigators found that NPY, which is naturally present in the central nervous system and the medulla, or core, of the adrenal glands, binds specifically to receptors on endothelial cells derived from adrenal blood vessels. The researchers suggest that NPY may produce constriction or relaxation of blood vessels depending on which one of two types of endothelial cell receptors, Y1 or Y2, it interacts with. These studies by the Puerto Rican scientists contribute greatly to understanding the possible roles played by NPY in regulating blood pressure.

Xavier Scientists Study Sugar Effects on Nerves, Muscles. Pharmacologists at Xavier University of Louisiana in New Orleans suggest that sorbitol, a natural glucose-related compound that accumulates slowly in nerve cells of diabetics, may be directly responsible for the numbness and loss of response to pain stimuli that occurs in advanced diabetes. The RCMI-funded scientists have shown experimentally that sorbitol decreases responsiveness to pain stimuli in rats.

Related investigations by the Xavier scientists, conducted in the toad *Bufo marinus*, have indicated that another glucose-related compound, inositol triphosphate, may act as an intracellular messenger in transmitting nerve signals that control the contractility of muscles in the stomach and other organs.

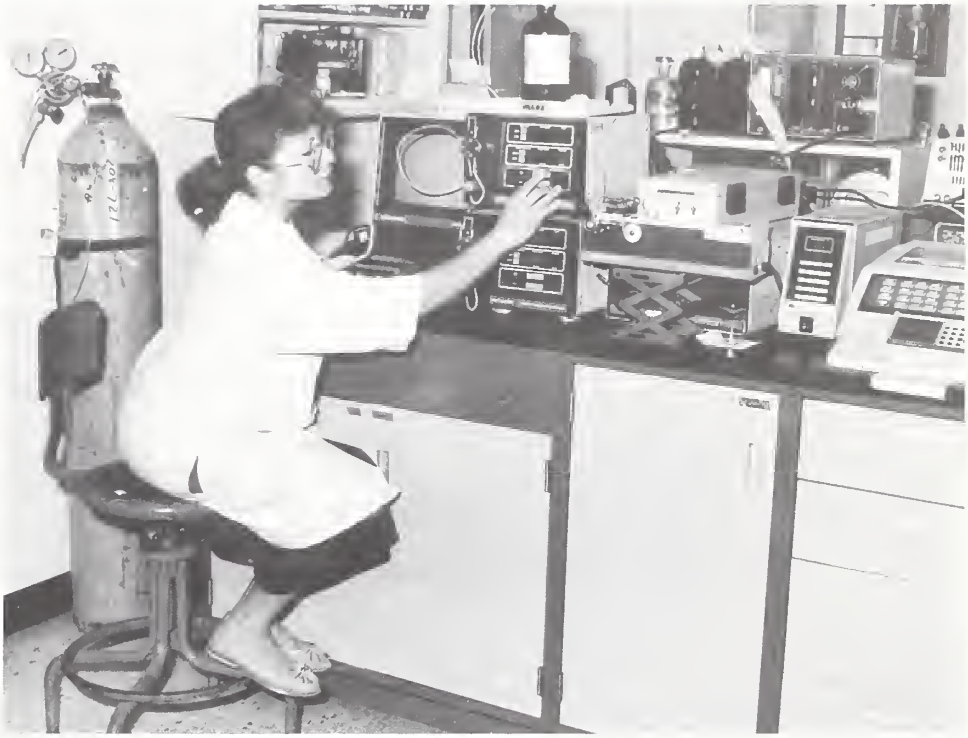
Two Genes Synthesize Reproductive Tract Hormone. A study of the genetic apparatus controlling the production of relaxin, a peptide hormone usually associated with dilation of the cervix during childbirth, has revealed that two different forms of relaxin are produced. Previous investigations had identified two different genes for relaxin, but only one of them seemed to be active.

RCMI scientists at the University of Hawaii in Honolulu reported that the two genes for relaxin were expressed not only in tissues from human placenta and breast but also in prostate glands. The RCMI scientists extracted messenger RNA from female and male tissue specimens; using the enzyme reverse transcriptase, they then produced its complementary DNA. A series of molecular biology experiments subsequently undertaken by the RCMI-supported group indicated, for the first time, that both of the genes H-1 and H-2 on human chromosome 9 are involved in synthesizing human relaxin.

Gene Regulation by Signal Transduction. RCMI-funded scientists at Hunter College of the City University of New York are studying the mechanism of signal transduction—transmission of signals from receptors on a cell membrane to genes in the cell nucleus. Signal transduction is essential to the hormonal activation of genes. New details on how gene activation works in various life forms were reported at an RCMI-supported conference held at the Center for the Study of Gene Structure and Function at Hunter College.

Dr. Robert P. Dottin, of the RCMI-funded Center for the Study of Gene Structure and Function at Hunter College, City University of New York, explores molecular changes occurring in slime molds during signal transduction, an important aspect of genetic activity.

Hunter College, City University of New York



Technician Rosa Flores works with sophisticated instruments for protein analysis at the University of Puerto Rico's Medical Sciences Campus in San Juan. The laboratory was established by the RCMI Program to support basic and clinical research and diagnosis.

University of Puerto Rico, San Juan

The scientists reported that reversible phosphorylation is an important part of the signal transduction pathway in slime molds. Reversible phosphorylation is believed to be a universal mechanism affecting hormone activation of a variety of different genes in organisms ranging from slime molds to humans. The New York City scientists identified a multigene family of phosphorylating enzymes by molecular cloning techniques.

Other investigators attending the symposium reported findings on signal transduction in intracellular and intercellular communications in mice, chickens, sponges, worms, and bacterial cells.

Computer Simulation of Schistosomal Disease Transmission. Computer scientists at Tuskegee University's RCMI in Tuskegee, Alabama, have applied computer technology and classic mass action theory to the epidemiology of the parasitic disease schistosomiasis. The Tuskegee scientists designed their computer modeling system to simulate the activities of various elements in the disease chain: humans that may become infected; aquatic snails that are hosts to the schistosome larvae; the schistosome itself; and the movement and contamination rate of waters by schistosome eggs.

Once the model can be shown to provide realistic approximations of parasite-snail-human interactions and infections, it is hoped that it will be useful in developing control strategies against this disease. Schistosomiasis is a health problem in 79 countries and affects more than 200 million people worldwide.

Tuskegee's RCMI team plans to apply additional health-oriented computer models to the battle against other disease problems. For instance, an effort is under way to develop a cardiovascular simulation model based on mathematical equations that describe the cardiovascular system as compartments in which flow rates, pressures, resistances, and compliances are dynamically related to the volume of blood in a living animal system.

Intramural Programs

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Veterinary Resources Program 50

Overview of Intramural Research Resources Actual Obligations

National Center for Research Resources Overview of Intramural Research Resources FY 1991 Actual Obligations

Program	Management Fund*	Service And Supply Fund	Total
Office of the Director/ Office of Animal Care and Use	$ 5,150,000	$ 669,000	$ 5,819,000
Biomedical Engineering and Instrumentation Program	$ 4,714,000	$ 8,472,000	$13,186,000
Library Branch	$ 4,854,000		$ 4,854,000
Medical Arts and Photography Branch		$11,337,000	$11,337,000
Veterinary Resources Program	$ 6,575,000	$13,503,000	$20,078,000
Total	$21,293,000	$33,981,000	$55,274,000

* Does not include reimbursements or charges related to space costs.

Biomedical Engineering and Instrumentation Program

Program Description

The Biomedical Engineering and Instrumentation Program (BEIP) contributes to advances in biomedical research through applications of engineering, mathematics, and the physical sciences. Approximately 40 physical scientists and engineers collaborate each year on more than 200 projects to produce advanced instrumentation, models, and techniques dedicated to the acquisition of biomedical information previously unavailable to NIH scientists. BEIP engineers have collaborated to produce advanced technologies that are now used throughout the world.

In addition, approximately 75 technical staff respond to about 1,400 requests for fabrication or major modification of laboratory devices and nearly 10,000 requests for repairs and minor modifications of scientific equipment.

Professional Engineering. BEIP's physical scientists and engineers perform independent research and collaborate with NIH intramural scientists on biomedical measurements, mathematical modeling, imaging, and the design of specialized instrumentation. The Professional Engineering staff members work within sections devoted to applied clinical engineering, chemical engineering, electrical and electronic engineering, and mechanical engineering. Specialty groups engage in pioneering research in magnetic resonance imaging and spectroscopy, digital image processing, analytical electron microscopy, and ultracentrifugation. BEIP also provides administrative and technical support to the NIH In Vivo NMR Research Center.

Scientific Equipment Services. BEIP instrument makers and medical and scientific equipment specialists construct, modify, repair, sell, and lease scientific equipment in support of NIH intramural research programs. These staff members produce sophisticated instruments that comprise electronic components, large or miniature mechanical components, polymers, and glass. BEIP offers NIH researchers a variety of comprehensive plans for maintenance and repair of scientific equipment and personal computers. BEIP's Scientific Equipment Resources Program sells and leases commonly sought scientific equipment, offering researchers shortened procurement times, flexibility in configuring their laboratories, and cost savings.

Research Highlights

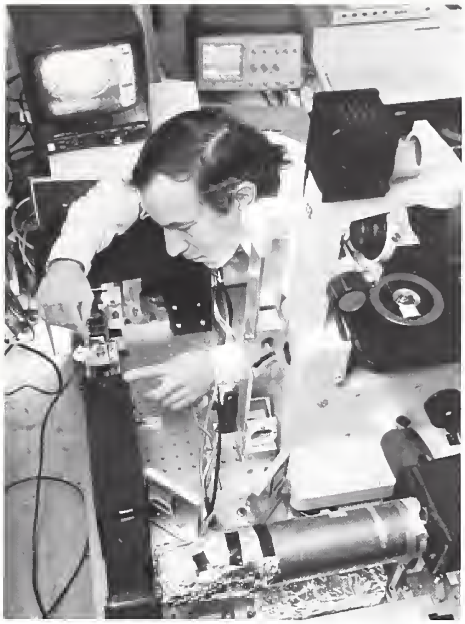
Disease Diagnosis

Mammography Scanner. The usefulness of mammography in early detection of breast cancer is well established. Current recommendations call for all women over the age of 40 to have a mammogram at regular intervals, but some women worry about exposure to x-ray radiation during mammography. Engineers in the Mechanical Engineering Section of BEIP have developed a new mammography instrument that reduces x-ray exposure two- to threefold while maintaining image quality.

The new instrument relies on a system of thin metal sheets called vanes that are located beneath the breast support and are invisible to the patient. These vanes move in a precise pattern and function to absorb scattered X rays. The device is able to produce a quality image with less x-ray exposure to the patient. The instrument is currently undergoing testing by the Food and Drug Administration.

Fiberoptic Sensors. The concentration of oxygen in blood and tissues of the body has important clinical implications. BEIP engineers in the Chemical Engineering Section are developing a fiberoptic sensor capable of measuring the concentration of oxygen in the body. Because it is very small and flexible it can measure oxygen in sharply defined locations such as the retina of the eye during eye surgery.

The fiberoptic sensor under development by BEIP engineers relies on a characteristic of oxygen called fluorescence quenching. Fluorescent light is produced by illumination of a dye placed inside the sensor. When the dye comes into contact with oxygen, the light is dimmed, or quenched. The amount of quenching is directly related to the amount of oxygen present in the tissue being sampled.



Dr. Seth Goldstein makes adjustments in his real-time confocal microscope used for optical sectioning, or slicing, of tissue.

William Branson, MAPB/NCRR

The fiberoptic sensor is capable of measuring oxygen continuously. Work done in anesthetized dogs has shown the probe to be practical and reliable. Plans are under way to use the fiberoptic sensor in clinical situations.

Tracking Eye Movements. Engineers in the Electrical and Electronic Engineering Section of the BEIP have developed a portable instrument capable of measuring and graphing eye movements. On a screen similar to a television screen a dot moves in a prescribed pattern. Patients are asked to follow the movements of the dot with their eyes, and their eye movements are recorded by an optical detector, connected to a personal computer.

The eye tracker is proving useful in diagnosing and monitoring the progression of diseases that attack the central nervous system. Because the control of eye movements requires a number of brain functions, researchers collaborating with BEIP wish to determine whether specific abnormalities in eye tracking may be useful in monitoring treatment of diseases like AIDS, schizophrenia, and Alzheimer's disease. Monitoring eye tracking ability over time may indicate whether and where disease progression is occurring. The device should also be useful in monitoring toxic effects of alcohol and other drugs on the brain.

Because the eye tracker is portable, researchers can bring the instrument to a hospital or to a patient's home for monitoring family members. The personal computer also allows data to be acquired and processed quickly and provides permanent data storage. New systems are being planned that will analyze the coordination of eye movements with hand responses and auditory stimuli.

Human Motion Analysis System. Studying human motion, particularly joint movements in three dimensions, has important clinical implications in designing prosthetic joints, diagnosing joint disease, and monitoring the efficacy of therapy. Obtaining information about joint movement in all three dimensions has been difficult, but a new system with major design contributions by BEIP engineers in the Electrical and Electronic Engineering Section is intended to help. The system relies on an improved television camera called a CCD camera, which is capable of detecting fast motion and is electronically stable. The CCD camera is coupled with strobe lights and computers.

Patients are fitted with reflectors on their joints and are asked to move in a certain place. When the strobe light hits a particular reflector, light is reflected back into the camera. The use of several reflectors, strobes, and cameras allows a three-dimensional image to be reconstructed by the computer.

Basic Science Research

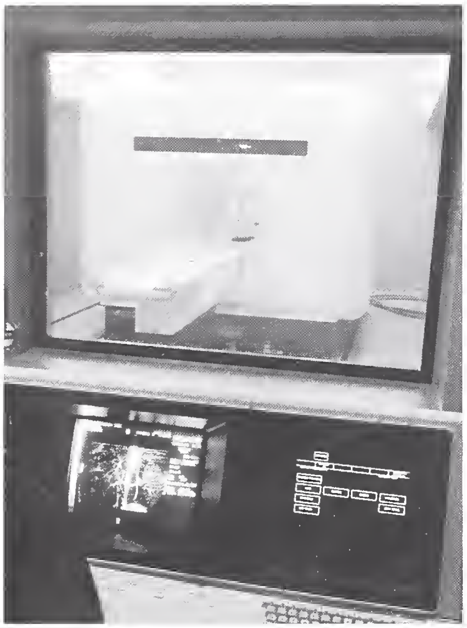
Thermodynamics of Lipid Bilayer Formation. Cell membranes in both bacteria and cells of higher organisms are composed of a lipid bilayer—a two-layer array of lipid molecules. How this bilayer forms and what makes it uniquely suited to compose membranes has been a subject of intensive study by cell biologists. BEIP engineers in the Applied Clinical Engineering Section are participating in a project that may help unravel the mechanism of cell membrane formation.

The engineers have designed a special calorimeter—a device that measures the heat generated in chemical reactions—capable of measuring extremely small temperature changes. Initial work with the calorimeter indicates that the temperature at which a bilayer will form is different for lipids extracted from different organisms. Now the researchers will try to determine exactly what the critical temperature of formation is for different animal cell membranes. This work may eventually help scientists to design drugs targeted to the cell membranes of specific organisms.

Confocal Microscope. As it looks beneath the surface of a specimen without physically cutting it, the confocal microscope produces three-dimensional images by combining optical "slices" of the object. Specialized optics reject light from all planes not in sharp focus so that background glare does not degrade the picture as in conventional microscopes. Recent advances in instrumentation have enabled BEIP engineers in the Mechanical Engineering Section to obtain real-time images of a variety of biological objects with the newly developed instrument.

The BEIP confocal microscope has no moving parts. This unique feature makes it possible to obtain pictures more rapidly than with other systems and allows the study of rapidly occurring biological phenomena. The new confocal microscope ensures ease of use by employing a personal computer to perform all alignment procedures and image generation.

Time-Resolved Spectroscopy. Time-resolved spectroscopy is a technique by which extremely rapid chemical reactions can be analyzed over very short time increments. Information about reaction rates and the sequence in a chain reaction can be obtained



The monitor of an imager in the NIH In Vivo NMR Research Center depicts results of an earlier experiment to visualize blood flow.

William Branson, MAPB/NCRR

using this method. Spectroscopy relies on the absorption of light by chemical reactants in different stages of a reaction. As a particular reactant changes, its pattern of light absorption also changes.

Innovations developed by BEIP engineers allow a high-resolution spectrum to be collected every 10 microseconds during a chemical reaction. This rate of data collection is about 1,000 times faster than is possible with any commercially available spectrometer. In addition, the instrument uses a laser to initiate the chemical reaction.

Time-resolved spectroscopy is being used to study the cytochrome enzymes, a group of several enzymes involved in transporting electrons in cells. The technique is suitable for studying any chemical reaction with reactants that absorb light.

In Vivo NMR Research Center. The In Vivo NMR Research Center is a centralized facility managed by the BEIP. Many researchers from different institutes and programs at the NIH, including BEIP engineers, utilize this state-of-the-art nuclear magnetic resonance (NMR) facility to conduct research on NMR applications.

The use of NMR to produce highly detailed anatomical images—through methods known as magnetic resonance imaging (MRI)—is well established in medicine. It is popular because of the quality of the images, and because it uses no harmful radiation. BEIP engineers are extending the use of NMR to study blood flow and metabolism, particularly in the central nervous system and in tumors. Through spectroscopic techniques NMR may be used for very early diagnosis of certain cancers by comparing the metabolism of normal cells and tumor cells.

Analytical Electron Microscope Facility. In cooperation with other researchers throughout the NIH, BEIP engineers are using the advanced electron microscopy known as parallel-detection electron energy loss spectroscopy (EELS) to determine locations of metal and other atoms in macromolecules. The presence and location of just a few atoms may be detected; in a demonstration of the technique the location of the iron atoms in hemoglobin, which carries oxygen in red blood cells throughout the body, has been shown. Other experiments have determined the molecular weight of large molecules.

This precise electron microscopy, for example, allows investigators to determine the location and number of calcium atoms within a nerve cell and perhaps to elucidate their function.

Dr. Richard Leapman uses the BEIP field-emission scanning transmission electron microscope to map structure and composition of cells and macromolecules at high spatial resolution.

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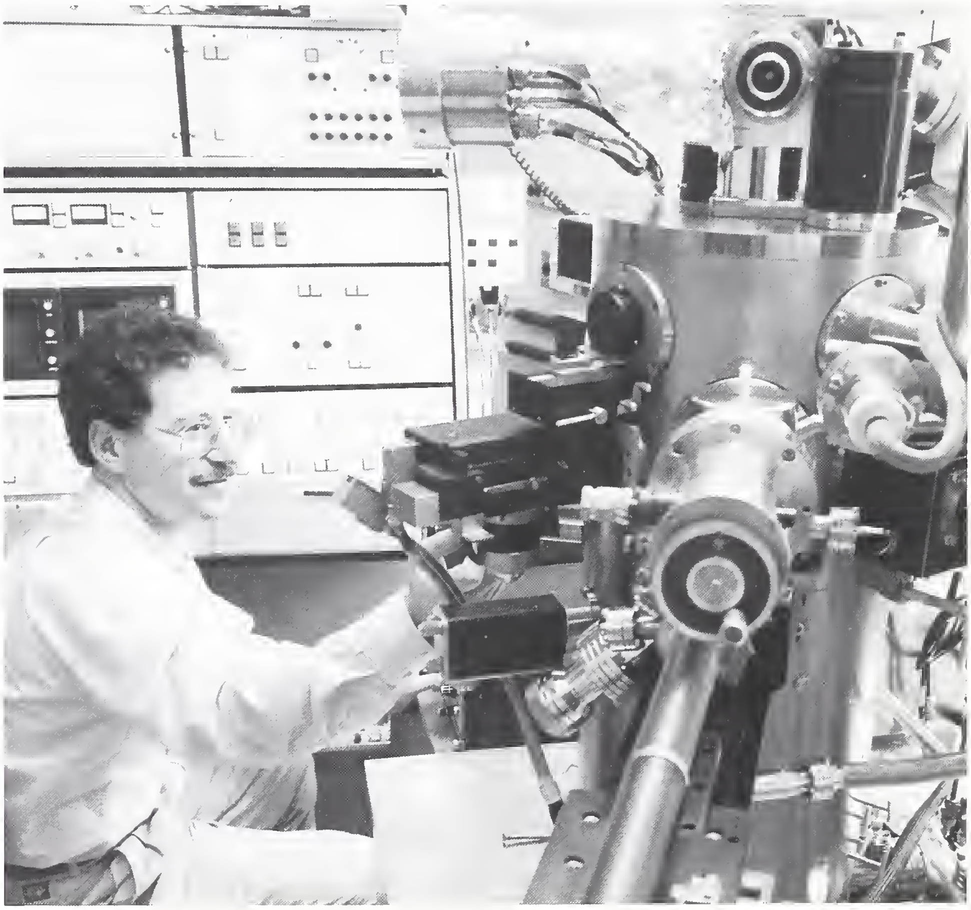

Library Branch

Program Description

With a collection of approximately 80,000 monographs, 155,000 bound periodicals, and 2,700 current journal titles, the NIH Library is one of the largest biomedical libraries in the country. Biomedical research fields comprise the majority of the collection, with physiology, psychology, biology, chemistry, and physics included. Small collections of interest to NIH administrative personnel are also maintained.

With such a large number of journals and periodicals in a fixed space, the NIH Library collection must be constantly weeded to make way for new information. Each year approximately 7,000 volumes are removed to make room for incoming material. Decisions on what to remove from the collection are based in part on information from the Library's online data collection system, which monitors the usage of journals. Subscription renewal decisions are also based largely on information available from the online system, which helps assure that the Library's holdings are of continued value to the NIH community. Each year a number of new subscriptions must be entered to adjust to program changes and to evaluate new titles. Concurrently, old subscriptions are reviewed to make certain they are still useful to the NIH staff.

The NIH Library has a 55-person staff, which is supplemented by contract staff for support functions. The range of services provided by the staff is diverse. It includes photocopying, a clinical library service, translation into English of scientific papers published in a foreign language, and compilation of bibliographies.

The photocopying service is well utilized. Approximately 4.5 million photocopies are made each year by NIH staff members for their own use and by library staff upon request. The vast majority of document requests—97 percent—are filled. Eighty-three percent are filled from within the NIH Library collection; the remainder of the requests are filled from other libraries such as the nearby National Library of Medicine.

Clinical librarians on the staff attend medical rounds and have developed special programs for AIDS, critical care medicine, and nursing education. Another librarian is a specialist in patent searching, and a third is an expert in bibliographic utility software.

The translation unit of the NIH Library is staffed by two translators, who fill the majority of requests. Approximately 20 percent of translations are prepared on contract.

Books are reshelved continuously during working hours, with approximately 650,000 volumes reshelved in a year. Most volumes are reshelved within 1 to 2 hours of receipt.

Librarians who specialize in library software applications offer advice and assistance in using software to manage small collections or create bibliographies. The Library also offers tutorials and classes in basic searching techniques using standard MEDLINE and Grateful Med, a program designed by the National Library of Medicine. The Library has access to more than 400 computerized databases, including those on the systems DIALOG, GenInfo, MEDLARS, BRS, STN, and Mead Data Central. The library staff performs approximately 1,250 computer searches each month. Selective dissemination of information service is also available to NIH staff. Approximately 450 searches are stored on SDILINE, TOXLINE, AIDSLINE, and CANCERLINE, with monthly bibliographies distributed.

In the reference area microcomputers are provided for NIH staff members to conduct their own searches. At one station MEDLINE files are available through the Grateful Med software. At another station a compact disc system allows searching of the latest 5 years of MEDLINE, with a tutorial to explain how to do so. These have proved so popular that several more stations are being acquired. The *Physicians' Desk Reference* is also available on compact disc, and the *Science Citation Index* is being added.

Changes at the NIH Library in 1991 included a new service to alert the NIH staff to the Library's excellent book collection. A bimonthly bibliography with a book display on a particular subject gave a visual reminder of resources usually obscured on the shelves. These displays were very popular and resulted in many requests for the books.

An improvement in the translation service was achieved in 1991. A simple change will

The NIH Library, which primarily serves the NIH intramural staff, has a collection of approximately 235,000 volumes and subscribes to 2,700 scientific journals.

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allow the cost of contract translation to be reduced considerably.

A local area computer network, installed in 1991, is currently being used by administrative personnel but will be connected to the catalog to make public access even more convenient.

The NIH Library is open 365 days a year. Although the library is intended as a resource for the NIH community, the public is welcome to use its extensive resources for research purposes. Carrels are available for patrons who require space and privacy for the preparation of speeches, papers, or other work. A library annex, located a few miles away, provides computer search services and reference services to local NIH staff.

Use of the NIH Library continues to increase. Each month more than 30,000 people enter the library, and 3,000 to 4,000 books are circulated. Journals do not circulate unless there is more than one copy of a particular title. The integrated computer system controls acquisitions, catalog, series, and circulation functions. Journal holdings also appear in the catalog, with daily receipts visible on screen. The number of people entering the library, the number of photocopy requests, and the number of items reshelved all bear testimony to the fact that the NIH Library provides a vital and well-utilized resource.

Medical Arts and Photography Branch

Program Description

The Medical Arts and Photography Branch (MAPB) produces a wide variety of visual documentation of scientific data, research programs, events, and accomplishments for NIH staff. Visual communications media produced by MAPB are used by NIH scientists in publications, exhibits, and presentations to the scientific community and the general public. MAPB's output of visual communications services is expected to exceed 1 million pieces of work this year, representing over 85,000 requests for services from within the NIH.

MAPB's staff includes professional artists, photographers, and audiovisual specialists who produce communication materials ranging from slides, viewgraphs and other projected visual aids to exhibit designs, statistical drafting, display charts, posters, medical illustrations, videotapes, and support for special events. This year four members of the MAPB staff received the NIH Director's Award for outstanding performance.

MAPB services are comparable in professional quality and price to commercially available services. To assure both quality and price, competing qualified vendors participate in daily bid sessions. A productivity study was conducted and completed in FY 1991, and proposals from this study will be reviewed and implemented in the upcoming fiscal year to improve MAPB's productivity.

To maintain and improve its high quality standards and meet demands for increased services, MAPB continues to update equipment. For example, the latest upgrades of Macintosh computers enable MAPB staff to offer a wide variety of computer-enhanced output to clients.

MAPB is divided into four sections: Graphics, Photography, Medical Illustration, and Design. The Graphics Section provides materials used for scientific presentations and publications, including illustrations, charts, graphs, tables, flow charts, slide presentations, and computer-generated graphics. The Special Events Unit within the Graphics Section provides support for one-time or unusual events or exhibits. One such event is the Medicine for the Public lecture series held each fall at the NIH. The Special Events Unit also produces signs for the NIH campus and NIH buildings, charts for Congressional hearings, and other projects.

Scientific technical photographer Richard Dreyfuss, of the Medical Arts and Photography Branch, works with Dr. Tiana Shiver using an inverted microscope. This instrument is used to image unstained cell cultures, which can be photographed in black and white or color, or placed on videotape for motion analysis.

William Branson, MAPB/NCRR

Video services provided by the Special Events Unit include inhouse videotaping, writing of scripts, production of shows used for public service announcements, video news releases, new drug therapy treatment videos, patient videos, and videotaping of NIH special events. Visual information specialists, exhibit specialists, and a TV production specialist work with clerical support within the section to provide these services.

MAPB's Photography Section provides central photographic services to the NIH community, including information, clinical and surgical photography, micro- and macrophotography, and photographic laboratory processing. Staff members use a diverse range of photographic facilities to accommodate the high volume of requests received each day. The Photographic Section is currently evaluating the demand for digitally enhanced electronic imaging for all photographic applications within the NIH.

The Medical Illustration Section produces a wide variety of black and white and color medical illustrations, as well as diagrams of surgical gross pathological, biological, and dental subjects. In addition, the Medical Illustration Section provides illustrations to be used in poster design, publications, exhibits, and newsletters. The section's long-term illustration project entitled "Atlas on Oncologic Surgical Techniques" is near completion. The remaining chapters on the head, neck, and limbs should be completed within the next year. A new project consisting of 50 medical illustrations for a monograph on vascular access techniques is in the beginning stages; the monograph will focus on topographical drawings and surgical procedures on veins.

The Design Section of MAPB provides publication design for public information projects, scientific presentations, conferences, editorial and technical illustration, and other services requested by clients. Its creative and talented designers produce award-winning posters, illustrations, and other printed materials that visually broadcast the NIH research mission.

Veterinary Resources Program

Program Description

The Veterinary Resources Program (VRP) provides professional and technical support to NIH intramural research efforts involving the use of laboratory animals. The program obtains, houses, and manages research colonies of nonhuman primates, dogs, cats, rodents, rabbits, and livestock. These research animals are used in studies conducted by the intramural programs of NIH institutes, centers, and divisions as well as by those of ADAMHA, FDA, and other Federal agencies.

The two branches of VRP each comprise three sections. The Research Animal Branch encompasses the Carnivore and Ungulate Section, the Primate Section, and the Rodent and Rabbit Section. The Scientific Services Branch includes the Genetic Resource Section; the Laboratory Sciences Section; and the Surgery, Radiology, and Pharmacy Section.

The program manages and operates central animal surgery and radiology facilities to aid scientists whose work requires these specialties. The VRP also has an animal health surveillance program that helps maintain the health of research animals by providing expertise in special diagnostics and disease control. In further support of biomedical research, VRP offers consultative services to NIH scientists and others throughout the world on the health, care, husbandry, genetics, and nutrition of animals.

The NIH Animal Genetic Resource (NIHAGR), managed by VRP, contains more than 300 rodent and rabbit strains, congenics, recombinant inbreds, stocks, and mutants that are maintained or being developed as models of human disease. Scientists from public and private institutions worldwide can obtain breeding nuclei from this resource. The NIHAGR is designated by the World Health Organization as a Collaborating Centre for Defined Laboratory Animals and by the International Council for Laboratory Animal Science as a Nude Mouse Repository.

Plans to renovate existing facilities for the housing and use of swine, dogs, rodents, and rabbits have been developed and implemented with the goal of optimizing the quality of animal space and maximizing its usefulness to NIH intramural scientists. VRP also plans and provides management and operational support to several new central animal facilities, one of which is a centralized rodent and rabbit facility that opened in FY 1991. Design phases are under way for new facilities for small ungulates and transgenic rodents and for renovation of existing primate and dog facilities. The VRP is currently involved in planning the management of an animal care program in a new research facility that will be shared by seven NIH institutes. The building is in the final phase of construction and is expected to be occupied by the fall of 1993.

Research Highlights

Animal Welfare

Steroid Measurement. The level of steroid hormones in the body may be a useful index of general health, integrity of the immune response, reproductive abilities, and response to stress in humans and animals. Measurement of body steroids—including adrenal and sex hormones—usually requires a blood test, but VRP researchers in the Primate Section are working on a noninvasive method for steroid measurement that involves collecting urine and fecal material.

The scientists measured the levels of cortisol and several different steroids related to reproductive function in the blood, urine, and feces of male and female baboons. The concentrations of the steroid metabolites were also determined. Baboons were fed a diet containing varying levels of fiber to determine if dietary fiber concentration influences the excretion rate of steroid metabolites, and longitudinal studies were performed to assess the influence of the reproductive cycle and pregnancy on body steroid levels.

Once a clear correlation between blood and fecal levels of steroids is established, it will be possible to devise procedures to assess the general health of captive animals. This correlation will also give researchers a method to measure the level of stress and reproductive condition of the wild animals being studied and may ultimately help to accomplish more successful breeding in captivity.

Biological laboratory technician Wendy Chittick is observing mice in the NIH Animal Genetic Resource and entering data on the Veterinary Resources Animal Data System. The animals are kept in a pathogen-free environment; workers wear sterilized outfits to avoid contaminating them.

William Branson, MAPB/NCRR

Viral Infection of Snakes. Snakes are being used with increasing frequency in scientific studies because they are relatively easy to maintain in a laboratory. However, not much is known about diseases that may affect laboratory snakes, particularly those that may be transmitted from one snake population to another.

The ophidia paramyxovirus infects a snake called *Lampropeltis calligaster*, or the prairie king snake. In the king snake the virus may cause severe pneumonia and pancreatitis. The virus also infects other species of snakes, including the rattlesnake, and was responsible recently for a number of fatalities among rattlesnakes in a large zoo.

VRP researchers in the Laboratory Sciences Section experimentally infected king snakes and brown tree snakes with paramyxovirus. Although the king snakes developed characteristic pathology associated with paramyxovirus infection, the brown tree snake did not. This apparent insusceptibility to paramyxovirus infection may make the brown tree snake better suited for laboratory work.

Epidemiology of Fatal Feline Respiratory Tract Infection. Despite careful management and inoculation procedures, animal colonies maintained in the laboratory may develop infections. A fatal respiratory tract infection in a population of cats at the NIH was due to a bacterial strain known as CDC Group EF-4. The infection was fatal for five cats, and over the next few months the entire colony had increased fatalities that were not directly attributable to the EF-4 bacteria.

The researchers investigated the EF-4 bacterial strain responsible for the outbreak and compared it to other strains. The pathology and medical histories of the cats were reviewed. The VRP scientists found that the EF-4 strain responsible for this outbreak was not the same as any control strain available, so it may represent a new mutation of the bacterium. By further studying this new strain, future outbreaks may be controlled and possible long-term effects of infection in humans and animals may be avoided.

Animal Models for Human Disease

HIV-Associated Nephropathy. As many as 10 percent of people infected with HIV, the AIDS virus, may also develop kidney damage. In these patients normal kidney function is impaired by sclerosis, or hardening, of the structures within the kidney known as glomeruli. Since the kidneys perform the essential function of filtering the body's waste products, HIV-associated glomerulosclerosis can be life-threatening.

In collaboration with researchers at the National Institute of Dental Research, VRP scientists have developed an animal model for HIV-associated kidney disease in transgenic mice. These mice contain a partial HIV genome.

The mouse model develops the HIV-associated kidney disease relatively early in the course of infection, and pathological examination of the kidney lesions shows they are very similar to those developed in people with HIV infection. The investigators hope to identify the mechanism whereby HIV infection produces kidney disease in the transgenic mouse model, and perhaps find a way to interrupt the disease progression. The information gained through this study may one day be applied to preventing or treating HIV-associated kidney disease in humans.

Hyperlipidemic Rabbits. A human disease called familial, or inherited, hypercholesterolemia results in very high blood cholesterol levels and frequently early atherosclerosis and coronary artery disease. Studies of similar diseases in animals may give researchers an indication of how coronary artery disease develops over time. The Watanabe heritable hyperlipidemic (WHHL) rabbit is an animal model for human familial hypercholesterolemia. Recent studies in the Laboratory Sciences Section characterized the serum cholesterol and triglyceride levels of WHHL rabbits. The scientists noted age-specific changes in serum cholesterol concentrations and also found a correlation between animal gender and serum triglyceride levels. The researchers now hope to evaluate how genetics influences blood cholesterol and triglyceride levels.

Two types of WHHL rabbits have been described previously: those with early development and high incidence of coronary artery disease and those with late development and low incidence of the disease.

Recent work has shown that the two types of WHHL rabbits can be differentiated by their lipoprotein types. The lipoprotein differences may explain why some rabbits develop atherosclerosis early. Testing the rabbits in the VRP colony for lipoprotein type will allow selective breeding of rabbits to produce progeny with planned characteristics.

Animal Genetics Studies and the NIH Animal Genetic Resource

Animal Model Development. When studying human disease, researchers often look for animals with a similar disease that can be used as a model for its human counterpart. By

Biological laboratory technician Shirley Brown and Dr. Lin Xi are working in the VRP embryo cryopreservation laboratory. By freezing embryos of research animals, valuable and often irreplaceable models for human diseases are preserved for future study.
John Crawford, MAPB/NCRR

studying the animal model, important clues to a disease's pathology and its mechanisms of progression can be found.

VRP researchers in the Genetic Resource Section are developing animal models of human diseases. Rats are being bred that have diseases similar to rheumatoid arthritis and non-insulin-dependent (adult-onset) diabetes mellitus. Rats susceptible to chemical addictions are also being developed.

Immunocompromised mice carry one or more genetic mutations that adversely affect the immune system. By combining mutations that are known to suppress different immunologic components, scientists can evaluate each component singly or in various groupings. These studies will further understanding of immunocompromising diseases such as AIDS.

Embryo Cryopreservation Program. As part of the NIH Animal Genetic Resource (NIHAGR), VRP scientists in the Embryo Cryopreservation Program collect and freeze embryos of valuable animals used in research, including rats, mice, and rabbits. Surprisingly, the investigators have found that the animals' genotype, or genetic makeup, is very important in determining how the embryos withstand the freezing and thawing processes.

Continuing studies have also shown that each species has distinct requirements with respect to procedures for stimulating ovulation and collection of embryos. Ancillary projects are looking at different cryoprotectants and enhancing methods for embryo freezing, thawing, dilution, and transfer to pseudo-pregnant females.

Mouse Embryo Reconstruction. Embryos of animals that have rare genetic types or are models for particular diseases may be difficult to maintain in surrogate mothers that do not have exactly the same genetic makeup. VRP scientists are developing methods to reconstruct very early embryos, called blastocysts, that will not be rejected by surrogate mothers.

The blastocyst is the stage of the embryo just prior to implantation in the uterine wall. At this stage the embryo consists of two layers: the inner cell mass, which will become the fetus, and an outer layer that will form part of the placenta. VRP investigators have used the inner cell mass of one species and the outer layer of another species to form a hybrid blastocyst. The blastocyst will then be implanted into a recipient female of the same species as that from which the outer cell layer was harvested.

So far, reconstruction of blastocysts has been successful. If the technique results in viable pregnancies, it will be useful for ensuring the reproductive success of certain research animals and possibly even aid in the conservation of some endangered species as well.

Genetic Profiles of Laboratory Rodents. Scientists in the Laboratory Sciences Section of the VRP are attempting to locate and identify specific inherited characteristics on the chromosomes of laboratory animals. Inbred strains of rodents are being used for this project.

By using a number of biochemical, immunological, and molecular biological techniques, in addition to standard genetic analysis, the researchers can generate a genetic map, or profile, of different inbred rat strains. This information allows scientists to monitor the propagation of laboratory animals and to detect problems if they arise. The genetic profile also helps investigators to plan breeding for selected characteristics and for greater usefulness in research.

Biological laboratory technician Billy Barnes is working with germfree, genetically defined laboratory rats in a flexible film isolator.
John Crawford, MAPB/NCRR

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